ECOLOGICAL ASPECTS OF HEPATIC MICROSPORIDIOSIS IN COASTAL WATERS WITH PARTICULAR REFERENCE TO THE HOST *TAURULUS BUBALIS*

by

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D E C L A R A T I O N

This work has not been accepted in substance for any other degree, and is not concurrently being submitted for any other degree.

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This is to certify that the work submitted here was carried out by the candidate herself. Due acknowledgement has been given to any assistance received.

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A B S T R A C T

Ecological Aspects of Hepatic Microsporidiosis in Coastal Waters with particular reference to the Host Taurulus bubalis by J. A. Stubbs.

Hepatic microsporidia were investigated from 5 species of fish, namely Taurulus bubalis (Euphr.), Crenilabrus melops, (L.), Gaidropsarus mediterraneus (L.), Ciliata mustela (L.) and Scophthalmus maximus (L.) and intramuscular microsporidia from 2 species, namely Taurulus bubalis and Cottus gobio L.. Detailed ultrastructural studies carried out on hepatic microsporidia from T. bubalis showed it to be a new species belonging to the genus Microgemma (Ralphs) and it is named here as Microgemma dunkerli sp. n. Although hepatic microsporidia from the other species of fish are included in Microgemma, insufficient data was available to determine whether these were synonomous with M. dunkerli or a separate species. Intramuscular microsporidia were identified as Pleistohora sp. and Pleistophora vermiformis (Leger) from T. bubalis and C. gobio respectively. Unsuccessful attempts were made to experimentally transmit spores by per os and intraperitoneal injection using a variety of experimental hosts. Viability studies indicated that spores survive for at least one month under controlled conditions. A detailed study of host/parasite relations was carried out at Portwrinkle and comparisons made with populations from Roscoff, Wembury, Widemouth and Aberystwyth. Prevalence and intensity of hepatic microsporidiosis in T. bubalis decreased markedly northwards probably due to temperature. Overall infection levels were at their highest in late summer and autumn, '0' group fish being the most severely affected. The main ecological events of the life cycle of Microgemma dunkerli are presented in the form of a flow diagram. It is hoped that this work will form the basis for an epidemiological model.

C O N T E N T S

I N T R O D U C T I O N

According to Lom (1984), 35 of the 85 protozoa [genera] recorded from marine teleosts are potential pathogens, microsporidia being among the most severe causing disfigurement, growth retardation and reduced reproductive capacity (Sindermann 1970). Microsporidia have also contributed to heavy economic losses in fisheries including that of the rainbow smelt (Nepzy and Dechtair 1972), the golden shiner (Summerfelt and Warner 1970), the North American pout (Fischthal 1944 and Sandholzer, Nostrand and Young 1945) and flatfish (Sprague 1977).

It would be expected therefore that these pathogens will present a risk to the development of mariculture where high stocking densities are maintained in cages in coastal waters. McVicar (1975) has already described increased transmission rates of Glugea stephani in farmed plaice. An assessment of disease potential of pathogens endemic in local fish species of coastal waters would appear to be an essential consideration in site selection for cage culture. That infections carried by local fish can lead to epizootics in farmed stocks has been described by Wales and Wolf (1955) who implicated a local cottid species in the spread of microsporidiosis in trout farms. The occurence of high levels of hepatic microsporidiosis in intertidal teleosts inhabiting coastal regions of S.W. Britain and Northern France, notably Taurulus bubalis and the rockling Ciliata mustela and Gaidropsarus mediterraneus, therefore gave cause for concern, for although these fish are not in themselves commercially important they are a potential source

of infection.

Although 94 species of microsporidia have been recorded from over 70 species of fish (Sindermann 1970, Sprague 1977) relatively little is known concerning their life cycle and ultrastructure. The aim of this study was first to describe and identify the causative agents of hepatic microsporidiosis in coastal fish and hopefully to determine the extent of host specificity in relation to infection risk to commercial species. Second to attempt to establish an experimental system to investigate transmission and assess viability and third to investigate the ecology of the infected hosts and their pathogens. It was hoped, depending on the amount of data obtained that the investigations may lead to the construction of an epidemiological model capable of predicting the effect of pathogens on fish populations together with methods of controlling them.

TAXONOMY

Balbiani (1882) first recognised the microsporidia as a discrete group within the protozoa, placing them in the class Sporozoa. This significant step, however, was based on only one known species described by Naegeli (1857), namely Nosema bombycis from Lepidoptera. Glugea anomola found in the teleost Gasterosteus aculeatus (Moniez 1887) is the earliest record of microsporidia from a vertebrate host. Thélohan (1891) recorded a Sporozoan from Myxcephalus scorpius which Gurley (1893) later named Pleistophora typicalis. In 1895 Thélohan added Taurulus bubalis, Lipophrys pholis and Pungitus pungitus to the list of host species without apparent reason. Thélohan (1892) proposed the first classification within the Microsporidia considering the group sufficiently like the Myxosporidia to include them in the subclass Cnidospora. This view was later supported by Gurley (1893) and Labbé (1899). Over the next ten years many species were discovered including further representatives from fish, notably Glugea lophii (Doflein 1899) from Lophius piscatorius, Glugea stephani (Hägenmuller 1899) from Pleuronectes platessa and Pseudopleuronectes americanus. Linton (1901) recorded Pleistophora species from Rhombus tricanthus as Sporozoa, which Woodcock (1904) later placed within the Microsporidia, and finally Léger (1905) recorded Pleistophora vermiformis from Cottus gobio.

By the early nineteen hundreds sufficient numbers of microsporidia had been described to enable reassessment

of families and genera. Perez (1905) redefined the families Nosematidae and Glugidae together with their genera and Stempell (1909) erected the family Pleistophoridae. Glugea hertwigi from Osmerus eperlaus was discovered by Weissenberg (1911) and included in his review with two previously described species from teleosts. Glugea branchiale was recorded by Nemeczek (1911) from fish at the market in Vienna. Notable publications in the 1920's include Debaisieux (1920), Shrader (1921) and Chatton and Courrier (1923). The latter recorded Nosema cotti from Taurulus bubalis. Léger and Hesse (1922) provided a classification scheme forming the basis of the comprehensive monologue 'A Biological and Taxonomic study of the Microsporidia' Kudo (1924). This remained an authority on microsporidian classification until the application of electron microscopy which Vavra (1968) amongst others considered to be an essential tool of the microsporidian taxonomist. Meanwhile many new species were recorded from teleosts including Nosema ovoideum (Raabe 1936) and Pleistophora hyphessobryconis (Shäperclaus 1941). A useful review by Poisson (1953) listed 40 species of microsporidia parasitizing fish. The order Microsporida was raised to class by Corliss and Levine (1963) although remaining in the subphylum Cnidospora. It was demonstrated by Sprague (1966) that there were no true affinities between Myxosporidia and Microsporidia and this led him to recognise both groups as subphyla within the Protozoa (Sprague 1970). Finally emphasizing the uniqueness of this group of parasites, Sprague (1977) and Weiser (1977) proposed elevation to the phylum status and Sprague (1977) introduced the term Microspora. At a recent meeting of the Society of

Protozoologists the taxonomy of protozoa was revised by the committee in order to emphasize the distinctness of eukaryotic organisms (Cox, 1981 Editor).

As far as the present study is concerned the first probable recording of a microsporidian from Taurulus bubalis was made by Thélohan (1895) who described a sporozoan from the musculature which he attributed to the species Pleistophora typicalis, previously assigned to the intramuscular sporozoan of Myxcephalus scorpius by Gurley (1893). Dunkerly (1920) briefly mentions the occurrence of hepatic microsporidia in the liver of Taurulus bubalis and assumed it to be Pleistophora typicalis. Canning and Nicholas (1980) have redescribed P. typicalis from the type species Myxcephalus scorpius and it remains to be seen if Pleistophora species in T. bubalis and M. scorpius are synonomous. A further species Nosema cotti was recorded from the testes of T. bubalis by Chatton and Courrier (1923). Recordings of microsporidia from the livers of teleosts at first appear relatively numerous and include the following, Glugea depressa and Glugea ovoideum. (Thélohan 1895) from Julis vulgaris and Motella tricirrata respectively, Glugea stephani (Hägenmuller, 1899) from Pleuronectes platessa. Pleistophora laborum recorded by Le Danois (1910) from Crenilabrus melops, Glugea hertwigi (Weissenberg 1911) from Osmerus eperlaus, Glugea machari (Jírovec 1934) from Dentex vulgaris, Nosema ovoideum (Raabe 1936) from Mullus barbatus, Glugea pseudofumefaciens (Pfugfelder 1952) from Brachydanio rerio. However some species are now regarded as synonomous and others are not exclusive to the liver as is the species under investigation in this study. Several

microsporidia have been recorded from freshwater sculpins including Pleistophora vermiformis (Léger 1905, Otte 1964), Pleistophora tahoensis (Summerfelt and Ebert 1969) and Pleistophora sp. (Wales and Wolf 1955). Since Naegeli (1857) first recorded Nosema bombycis recent reviews (Sindermann 1970, Sprague 1977) have listed over 94 species from 77 genera of fish alone. Recently at least another dozen species have been added to the list, including Tetramicra brevifilum (Matthews and Matthews 1981), Loma sp. (Morrison and Sprague 1981, 1983), Microgemma hepaticus (Ralphs pers.comm. 1981), Glugea truttae (Loubés et al 1981). Pleistophora sp. from Priacanthus sp. (Hua-Ding-ke, Dong Han-Ji 1983).

MORPHOLOGY

Until 1950 all species descriptions were based on light microscope studies and many of these not surprisingly provide insufficient data to enable definite identification. Host and site of infection, together with spore dimensions were the main criteria used in early classifications of Weissenberg (1911), Legér and Hesse (1916), Kudo (1924), Chatton and Courrier (1923) and Shäperclaus (1941). Such evidence was found to be inconclusive by Weiser and Colozzi (1972) when cross infections between different hosts showed variability in spore dimensions. The importance of the electron microscope in assessing taxonomic relationships within the microsporidia has been discussed by Vavra (1968), Loubés and Maurand (1975) and Canning (1977). The majority of early electron microscope studies, notably Steinhaus (1951), Krieg (1955), Weiser (1959), Huger (1960), Puytorac (1962), Kudo and Daniels

(1963), Vavra (1965) and Ishihara (1968) are chiefly concerned with studies of the microsporidian spore. The late 1960's saw interest developing in electron microscopy studies of teleost microsporidia. Important fundamental studies of microsporidian developmental stages, although not of fish are given by Weidner (1969, 1972), Szollosi (1971), Canning and Sinden (1973), Maurand and Vey (1973).

Electron microscope studies of microsporidia parasitizing fish, particularly marine species are relatively few (Canning, Hazard and Nicholas 1979, Canning and Nicholas 1980, Kinkelin 1980, Morrison and Sprague 1981, Matthews and Matthews 1981, Canning, Lom and Nicholas 1982). Most studies are concerned with transmission electron microscopy although the use of scanning electron microscopy has been investigated by Lom and Weiser (1972) and Larrson (1981). Serological and biochemical techniques have also been applied to taxonomy by Kalalova and Weiser (1973), Knell and Zam (1978), Streett (1979), Fowler and Reeve (1974 a,b).

DEVELOPMENT WITHIN HOST CELL

A great deal of literature has been written on the development of microsporidia since the application of electron microscopy. Vavra (1976 a) produced a detailed and comprehensive review up until 1976. A brief treatment of studies interpreting development of microsporidia within the host cell, relevant to the present study is given below. Weissenberg (1921, 1922), Kudo (1924), Weiser (1961) made important contributions to the understanding of developing microsporidia, before the use of electron microscopy in this field. However it is now accepted that the electron microscope is a necessary tool when investigating the life

cycle of microsporida (Vavra 1968). Sprague and Vernick (1966) and Lom and Corliss (1967) were the first to carry out ultrastructural studies of developing microsporidia within the teleost hosts, looking at Glugea sp. and Plistophora hyphessobryconis, respectively. The life cycle is divided into two phases, merogony and sporogony (Vavra 1976 a). The sporoplasm, the first stage in the life cycle is injected into the host from the spore via the extruded polar filament. Until Ishihara (1968) and Weidner (1972) demonstrated this phenomena using electron microscopy, a great deal of speculation surrounded it. Weidner (1972) also showed that reorganization of cytoplasm took place within the sporoplasm on contact with that of the host. The sporoplasm by vegetative reproduction becomes a meront. At electron microscope level the sporont is usually distinguished from meronts by extramembraneous deposits of electron dense material (Overstreet and Weidner 1974), or the laying down of an electron dense membrane around the sporont (Cali and Briggs 1967). Canning, Lom and Nicholas (1982) however found the opposite to be the case with Glugea anomøla with the meront being distinguished by extramembraneous deposits. Meronts may be cylindrical as in G.anombla, dividing by constriction of cytoplasm surrounding several nuclei to give a more rounded plasmodium (Canning, Lom and Nicholas 1982). They are structurally simple cells (Vavra 1976 b) with the surrounding cytoplasm often rich in mitochondria and lipoproteins which Sprague and Vernick (1968) and Loubés, Maurand and Walzer (1981) have suggested nourish the developing stage. Cisternae are visible (Sprague and Vernick 1968) along with smooth endoplasmic reticulum and loosely scattered

ribosomes, but no golgi body (Lom and Corliss 1967). The course of sporogony varies between species of microsporidia and is now used as a taxonomic feature (Sprague 1977), Debaisieux (1922) defines the sporont as the final division giving rise to sporoblasts. The sporont nuclei may divide once or several times. It has been suggested by Sprague (1977) that the first division is meiotic and proceeding divisions mitotic. Divisions of the microsporidian nucleus proceeds as acentriola pleuromitosis according to Hollande (1972). The "centriola plaque" (Sprague and Vernick 1977) or " spindle plaque" (Moens and Rappont 1971) serves as attachment for intranuclear spindle apparatus.

Several genera including Encephalitozoon, Nosema (Cali, 1971) and Icthyosporidium (Sprague, 1965) can be recognised by the immediate cytokinesis following the first nuclear division, which provides two future sporoblast cells. In other genera there are several divisions of the sporogonial nucleus before cytoplasmic separation, which produce various sized plasmodia. Future sporoblasts are pinched off by constriction of cell membrane, this causes variable members of sporoblasts to be formed from each sporont. Thelohania sp. always produce eight sporoblasts (Gassoumer and Ellis, 1973) whereas Pleistophora sp. and Glugea sp. have variable numbers and are polysporoblastic (Canning, Lom and Nicholas 1982). The presence of diplokarya or uni-nucleate meronts and sporonts is another important feature used by taxonomists. Kudo (1930) describes the four possible combinations. Nosema bombycis has diplokarya throughout its life cycle (Cali 1971, Youseff and Hammond 1971), Glugea sp. and Pleistophora sp. have no diplokarya

in their life cycles, Canning, Lom and Nicholas (1982) and Lom and Corliss (1967) respectively are but two combinations. Two types of sporont, giving rise to two kinds of spores have been recorded from Pleistophora typicalis and Pleistophora littoralis (Canning, Hazard and Nicholas 1979, Canning and Nicholas 1980). The sporoblast is defined as the stage immediately preceding the spore and the result of sporogony, the final division of the sporont. Canning, Lom and Nicholas (1982) while investigating Glugea anomola, regarded the stage prior to this division also as sporogony which gives rise to a mother sporoblast which in turn divides to give two sporoblasts and is therefore polysporoblastic. Loubés, Maurand and Walzer (1981) consider only the last division of the sporont in Glugea truttae as sporogony.

The sporoblast is considerably larger than the developing spore and has dense cytoplasm which produces future organelles. Sprague, Vernick and Lloyd (1968) and Sprague and Vernick (1966) held the view that the polar filament was of nuclear origin but more recently Sprague and Vernick (1969) and Szollozi (1971) found that the polar filament and its associated organelles were derived from the golgi apparatus. Sprague, Vernick and Lloyd (1968) have been able to pinpoint areas of future polaroplast formation. The last event of spore morphogenesis is rapid and concerns the formation of an endospore layer (Vavra 1976 b), Canning, Lom and Nicholas (1982) have shown that multisporous pansporoblastic forms attributed to the genus Pleistophora belong to at least three genera, by investigating intracellular development.

The majority of microsporidian studies today, particularly of new species include electron micrographs of developing stages (Canning, Hazard and Nicholas 1979, Canning and Nicholas 1980, Matthews and Matthews 1981, Morrison and Sprague 1981, Loubes, Maurand and Walzer 1981, Canning, Lom and Nicholas 1982).

FISH LIVER

The microsporidia under investigation in this study occurs in the hepatic tissue, hence it is necessary to investigate the structure and function of the liver. The majority of liver studies are carried out on mammalian tissue, but the following authors have looked at teleost livers, Weis (1972), Hinton and Pool (1976), Chapman (1981), Simon, Dollar and Smuckler (1967) and Eurell and Haensly (1982). The fluctuation of lipid during reproduction was studied by Peute et al (1978). Léger and Denton (1976) and Schiffman (1959) looked at lipid content of various teleosts. MacKenzie (1981) investigated the effect of a protozoan namely Eimeria in the liver of blue whiting and found liver weight as percentage of body weight decreased with increased infection. Hawkins, Solangi and Overstreet (1981) looked at the damage caused by another Eimerian E.funduli to the liver of killifishes but were unable to assess its effect on hepatic function.

EPIDEMIOLOGY

Most epidemiology studies, particularly early ones, are concerned with parasitic diseases of medical importance, notably malaria (Ross 1911, McDonald 1957, Gillies 1972), shistosomiasis (McDonald 1965, Ansari 1973, Barbour 1981)

and diseases of domestic animals including babesiasis ((Joyner and Donnelly 1979) and facioliasis (Wilson, Smith and Thomas 1982).

May and Anderson (1979a, b) proposed various epidemiological models which enabled theoretical predictions of host population changes due to disease. These were proposed on the basis of available data on viral and bacterial infections in laboratory experiments, enabling all parameters to be measured or controlled. Detailed knowledge of the host population or populations is essential in any epidemiological study, such studies of fish pathogens particularly in marine teleosts however are sparse. MacKenzie (1981) investigated an incidence of Eimerian infection in populations of blue whiting. Kennedy (1975, 1977) has studied ecological aspects of cestodes in fresh water teleosts. The only epidemiological study of microsporidiosis was carried out by Thomson (1958) in an insect host. However many studies of microsporidiosis have been carried out investigating one or two aspects of epidemiology. Stunkard and Lux (1965), Delisle (1969), McVicar (1975) and Olson (1976) found that temperature fluctuations controlled intensity of infection. Summerfelt and Warner (1970) and Chen and Power (1972) found that high infection in reproductive organs caused decreased fecundity. Geographical variation too has been recorded by Stunkard and Lux (1965), Summerfelt and Warner (1970) and Chen and Power (1972).

Epidemiology is a quantitative science, Anderson (1982) and relies on accurate measurement of all parameters which can then be used to form or fit existing models (Crofton 1971, Pennycuick 1971a, b, c). The theory and reality of

of epidemiological studies is discussed by Bradley (1982).

Fish host

Taurulus bubalis, the host under investigation in this study is an inshore fish occurring in the estuaries and rockpool habitat. Little work has been published on its ecology and population structure although Lamp (1966) provides a comprehensive work on a population in the Baltic. Holt (1887) and Russel (1935, 1938) made detailed studies of its larval and spawning stages and feeding habits have been investigated by Western (1968). Anatomical measurements for taxonomic purposes were carried out by Sandercock and Wilimovsky (1968) for all members of the Cottid genus, Enophrys.

Studies on other fish in the same environment as Taurulus bubalis such as Ciliata mustela, Gaidropsarus mediterraneus, Lipophrys pholis and Crenilabrus melops include those by Day (1880), Russel (1976) and Wheeler (1969). Studies of the rockpool environment, a habitat of extreme variability in temperature and salinity have been carried out by Klugh (1924), Daniel and Boyden (1975) and Stubbs (1980)

Transmission

Canning (1971) reviewed transmission of microsporidia in both insects and other invertebrates. The first microsporidia were transmitted to insects experimentally by Payne (1933). Bückmann (1952), Weissenberg (1968), Lom (1969), McVicar (1975), Olson (1976), Scarborough (1979) and Matthews and Matthews (1981), all successfully transmitted microsporidia in fish using direct and 'vector' methods. Stunkard and Lux (1965), Summerfelt and Warner

(1979) and Delisle (1969) failed to do so and the latter regarded physiological differences between populations as possible reasons for this. The longevity of spores (Kramer 1970) and their resistance to environmental factors (Maddox 1973, Brooks 1982) are important when considering 'infectivity potential' of the disease. Transmission experiments again on insect hosts have shown timing and concentration of spores to be a critical factor (Henry et al 1973). Ishihara (1968) found the extended polar filament from Nosema bombycis could pierce a cell wall and invade within seven minutes. However not all extruded filaments produce infections (Oshima 1973), although Bailey (1972) showed that one spore, if virile can produce an infection. Laboratory transmission provides a convenient method for studying intracellular development of microsporidia (Weissenberg 1968). Various methods of spore extraction can be used according to Cole (1970), Weidner (1972), Hostounsky (1978) and Kelly and Knell (1979).

Temperature was found to be an important factor in regulating infection and hence transmission (Stunkard and Lux 1965, Delisle 1969, McVicar 1975, Olson 1976 and Takvorian and Cali 1981). Where microsporidia infect reproductive organs, Summerfelt (1964), Chen and Power (1972), Summerfelt and Warner (1970) have found that fecundity will decrease with higher infections. Olson (1976) and Burn (1980) found young fish particularly susceptible to infection. Populations of fish occupying different geographical locations have been found to support varying degrees of infection (Stunkard and Lux 1965, Summerfelt and Warner 1970 and Chen and Power 1972).

Figure 1a. Seaward view of the shore at Portwrinkle at low water springs. Note beacon, approximately 5 metres high at extreme low water springs.

Figure 1b. Typical rockpool exposed at mid-tide level from which fish were collected. Note abundant *Fucus* and *Corallina* sp.

MATERIALS AND METHODS

FISH

A total of 453 fish from 10 stations (Table 1) in south west Britain and northern France have been examined for hepatic microsporidiosis. These were collected between November 1980 and May 1983. Portwrinkle (Figs.1,2) was selected for a detailed ecological study because of its close proximity to Plymouth, easy access to the foreshore and relatively large samples which suggested high levels of infection with hepatic microsporidia. Data from the remaining stations enabled a more extensive study to be made on the geographical distribution of parasites in Taurulus bubalis. In addition, it was possible to identify a population with a low incidence of infection at Aberystwyth, which could be used as a source for experi mental studies in transmission. The majority of fish examined in the survey were Taurulus bubalis (Euphrasen, 1786) (fig.3), however other rockpool fish namely Crenilabrus melops (Linneaus, 1758) (fig.4), Ciliata mustela (Linneaus, 1758) (fig.5), Gaidropsarus mediterraneus (Linneaus, 1758) (fig.6), Lipophrys pholis Linneaus, 1758, Callionymus lyra Linneaus, 1758 and Gobius species were also examined.

Collection

Rockpool fish were caught using hand nets following anaesthetization with a solution of 20% quinaldine in acetone (Bagenal 1973). On collection the fish were immediately placed in fresh aerated sea water and transported alive to the laboratory where they were maintained

Table 1. To show sampling sites in south and west Britain and northern France together with fish species and their numbers caught

STATION	MONTH AND YEAR OF COLLECTION													SPECIES AND NUMBER CAUGHT											
	January	February	March	April	May	June	July	August	September	October	November	December	Year	Callionymus lyra	Chelon labrosus	Ciliata mustela	Cottus gobio	Crenilabrus melops	Gaidropsarus mediterraneus	Gobius flavescens	Gobius niger	Lipophys pholis	Scopthalmus maximus	Taurulus bubalis	TOTAL
1. Aberystwyth								✓				✓	81											14	14
								✓					82			1								11	12
2. Gower								✓					82					1	2			2			5
3. Widemouth											✓		80											3	3
										✓			81			3								5	8
													83			3			1		1			9	14
4. Newquay					✓								81											1	1
5. Portleven	✓	✓											81									8			8
6. Portwrinkle	✓				✓	✓	✓		✓	✓	✓	✓	81	1		8		2	3	1		2		66	83
		✓	✓	✓	✓	✓	✓	✓		✓	✓		82					3	3			1		142	149
		✓	✓	✓									83			5		2	2			2		33	44
7. Wembury		✓		✓	✓								81			4		3	2	1	1			20	31
			✓				✓	✓					82					2						18	20
8. Ringmore								✓					81			2								4	6
9. Prawl Point								✓					81			1									1
10. Roscoff									✓				82	1				2	2		2			21	28
11. St. Johns				✓									81		10										10
12. R. Lynher					✓								81				28								28
13. Shillamill Lakes							✓						82				50								50

Figure 2 Coastal map of S.W. Britain and Brittany to show collection sites for fish

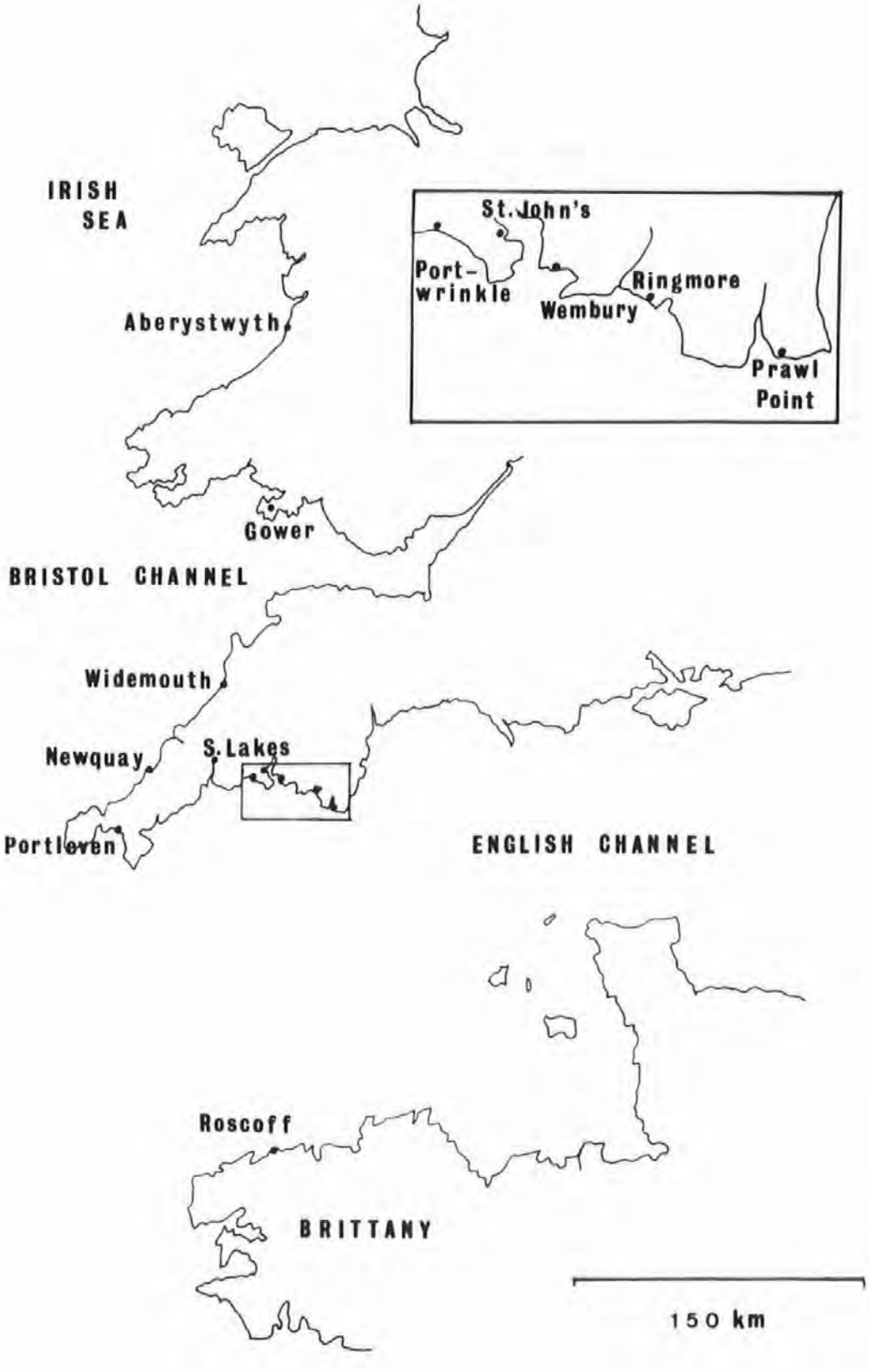
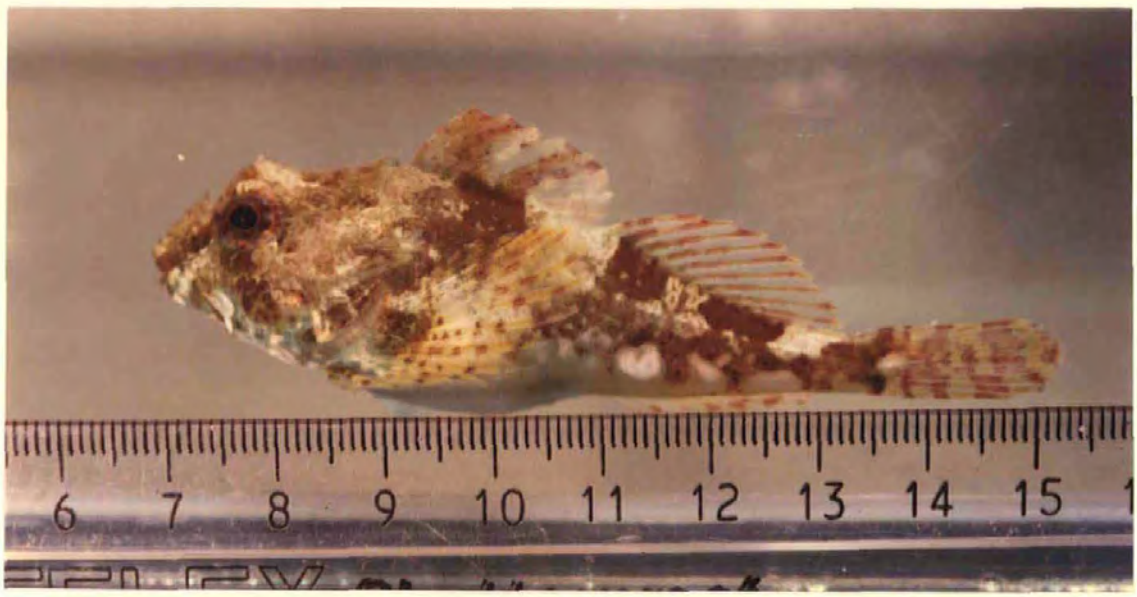

IRISH
SEA
St. John's
Port-
wrinkle
Ringmore
Wembury
Prawl
Point
Aberystwyth
Gower
BRISTOL CHANNEL
Widemouth
Newquay
S. Lakes
Portleven
ENGLISH CHANNEL
Roscoff
BRITTANY
150 km

Figure 3 Taurulus bubalis (Euph)

Figure 4 Crenilabrous melops (L)


6 7 8 9 10 11 12 13 14 15


cm

Figure 5 Gaidropsarus mediterraneus (L.)

Figure 6 Ciliata mustela (L.)


cm


cm

in aquaria. 'O' group turbot were collected using a push net at low tide. Juvenile mullet were seine netted at St.Johns Lake and Cottus gobio Linneaus, 1758 the fresh water sculpin, caught using hand nets from freshwater streams entering the River Lyhner at stations 11 and 12. The latter species were collected for possible use as experimental hosts in transmission experiments due to their close taxonomic relationship with Taurulus bubalis, being in the same family Cottidae.

Aquarium maintenance

Stocks of adult rockpool fish were kept in polypropylene tanks (40 x 30 x 80 cm) at stocking densities not exceeding 10 fish and juvenile fish kept in small perspex tanks (24 x 26 x 42 cm) at similar stocking levels. Water quality was maintained in large tanks by an Eheim biological filtration unit, filled with shell gravel and in small tanks by under gravel filtration, water being circulated by means of a Reno air pump. Temperature was controlled in large tanks by passing returning filtered water through a Buswell cooling unit, regulated by a thermostat. Water temperature in small tanks was maintained by placing them in a water bath at the required level. Sea water for the marine aquaria was obtained from the research supply of the Marine Biological Association, the quality of which is constantly monitored for salinity, pH and pollution. Particular attention was given to the quality of water used in transmission experiments to decrease the risk of adding infective agents from the environment. The water was allowed to stand for a week to allow spores and debris to settle and then the supernatent was passed through a

millipore filter of 0.35 µm pore size. To increase filtration the apparatus was attached to a simple water vacuum pump. Aquaria were checked daily and debris removed. A partial water change was carried out at least every two weeks and a complete change every one or two months depending on water quality. Nets were kept separate for each tank and frequently sterilized in Milton. Tanks were sterilized before transmission experiments were undertaken. Different feeding regimes and types of food were required depending on the age and species of the fish. Juvenile Taurulus bubalis were fed on Artemia and tubifex worms while juvenile Cottus gobio were fed on Artemia and Daphnia. Adult rockpool fish and turbot were fed on pieces of white fish and adult Cottus gobio fed on tubifex worms. Juvenile fish were fed twice and adult fish once a day. Uneaten food was removed after an hour.

Post mortem

Fish were examined immediately following death where possible and preferably on the same day of capture. They were killed using an overdose of MS 222 (Ethyl-m-amino benzate). A systematic examination was carried out as follows, data being recorded on a case card as shown in Fig.7. Prior to opening the fish its external condition, length (head to caudal peduncle) and total body weight were noted. The body cavity was opened by means of a ventral incision and the sides carefully cut away to reveal the viscera including the liver. The foci of microsporidian infection (Fig.8 a,b) could be clearly seen with the aid of a Kyowa dissecting stereo microscope. The translucent nature of a fresh liver enabled most foci of established

Figure 7 Case card designed for recording ecological data in Fishes

CASE CARD REFERENCE NUMBER _____ DATE _____

TIME _____ STATION _____

TIDAL STATE: | SPRING | NEAP | MID |

SHORE LEVEL: | LWS | LWN | MTL | HWS |

ENVIRONMENTAL DETAILS: SEA TEMPERATURE ^{o}C _____

HOST DETAILS: SPECIES _____

LENGTH (cm) _____ WEIGHT (gm) _____

AGE GP.

SEX | F | M | IMM |

GONAD WEIGHT (gm) _____

APPEARANCE | NORMAL | EMACIATED | LESIONS |

MICROSPORIDIAN INFECTION:

LIVER: WEIGHT (gm) _____

APPEARANCE			LEVEL INFECTION		
LIGHT	DARK	BLOTCHY	LOW	MEDIUM	HEAVY

OTHER SITES OF MICROSPORIDIAN INFECTION:

GUT _____ SPP _____

GONAD _____ _____

MUSCULATURE _____ _____

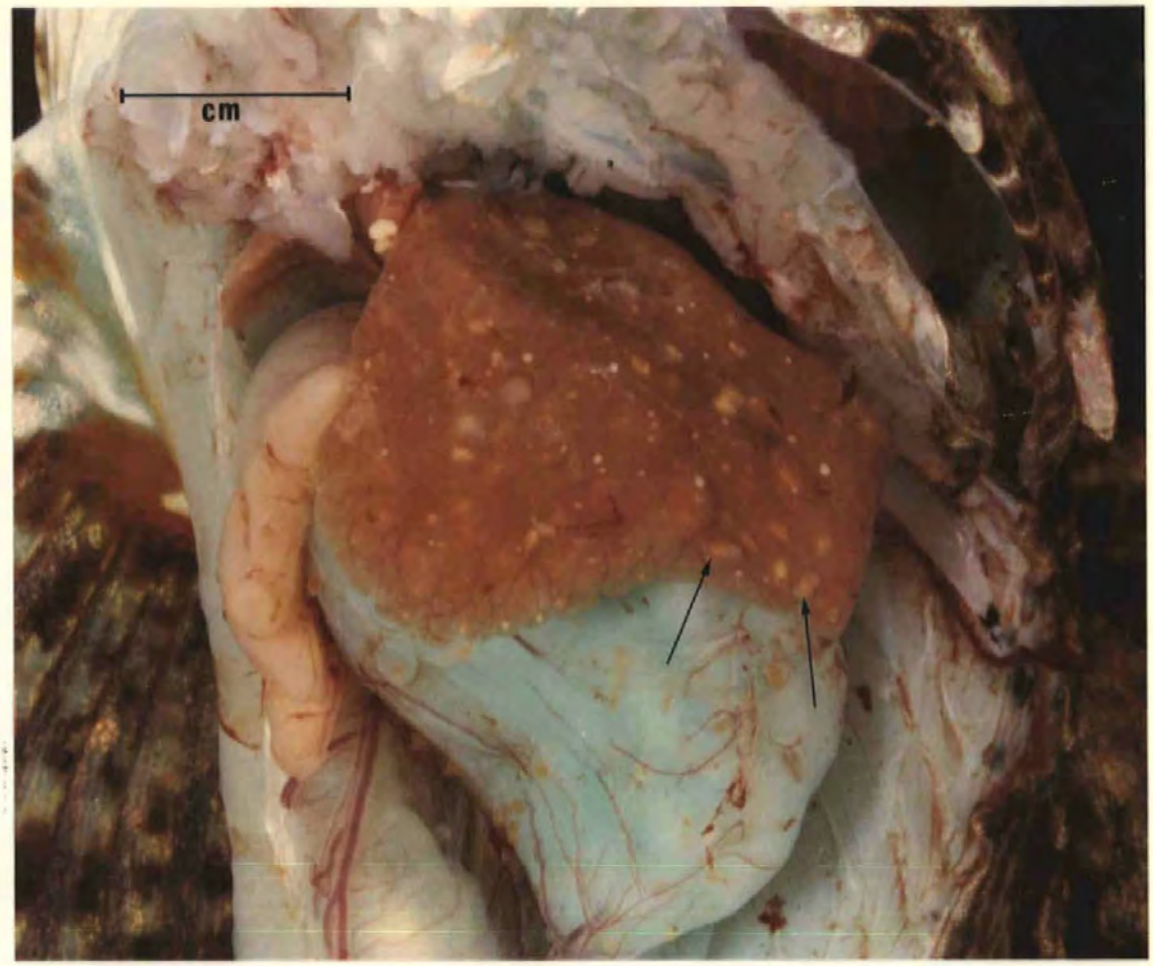
OTHER PARASITES PRESENT IN LIVER TISSUE:

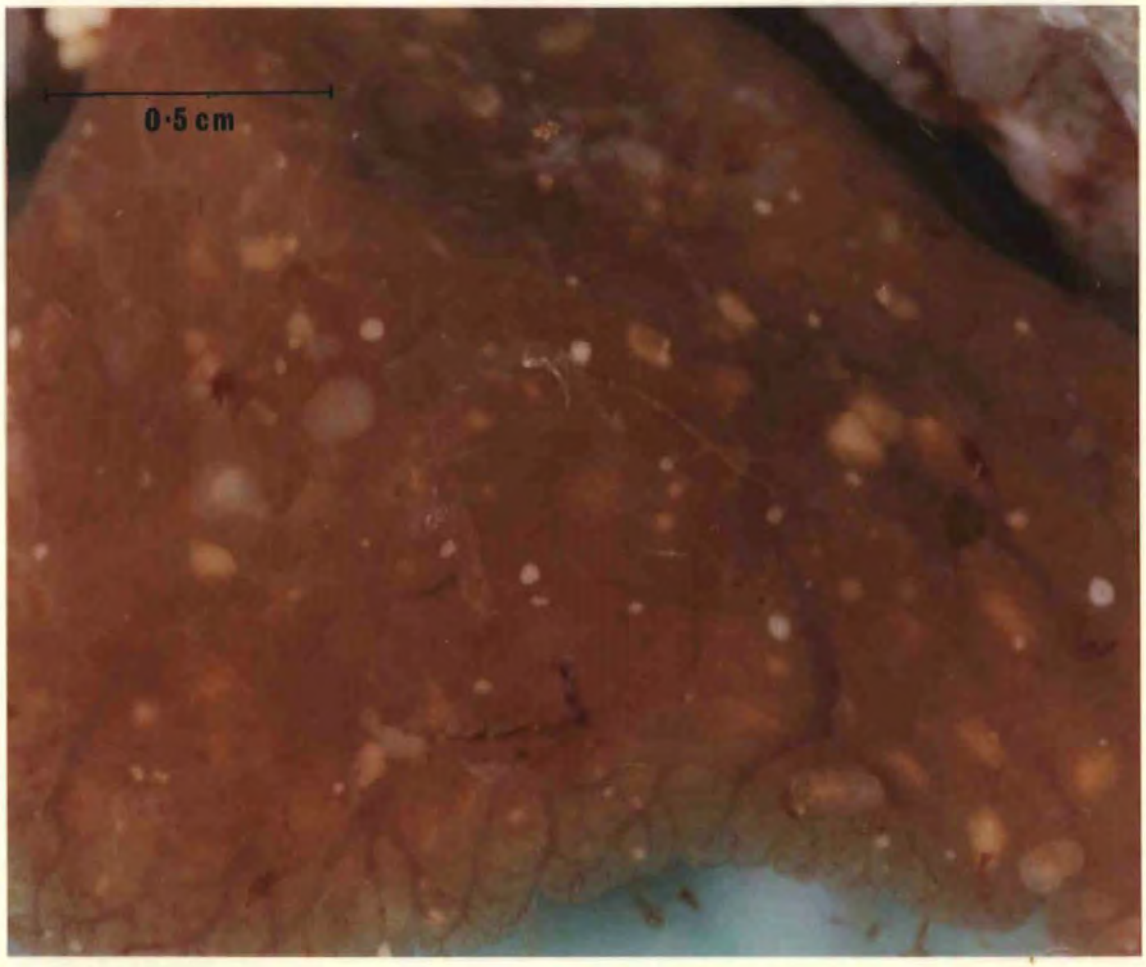
MICROSPORIDIA			METACERCARIA			JUVENILE NEMATODE		
L	M	H	L	M	H	L	M	H

Infection: L = low M = medium H = heavy

Figure 8a Late 'O' group Taurulus bubalis open to show liver infected with hepatic microsporidiosis. Note also encapsulated metacercaria (arrowed).

Figure 8b Surface of liver at higher magnification to show irregular shapes and sizes of focal points of infection.


cm


0·5 cm

infection to be clearly identified as isolated white areas within the liver and a simple assessment of infection level was based on number present as follows.

Absent	Low	Medium	Heavy
0	1-20	21-70	71+

Two other types of parasites were recorded from the liver of Taurulus bubalis namely the hemiurid metacercaria and the occasional larval nematoda. The following scale was used to assess infestation with the metacercaria.

Absent	Low	Medium	Heavy
0	1-10	11-30	30+

The fish was then examined for other microsporidian infections, particularly those infecting the musculature. The liver was then carefully removed and further checks made before weighing and processing for lipid estimation. The gonads were removed, weighed and examined for signs of microsporidiosis by squashes and histological sectioning. Otoliths were dissected and cleaned in light oil and the age of the fish determined.

Quantitative determination of total lipid within the liver of Taurulus bubalis

Total Lipid was determined colormetrically using a total lipid test (Boehringer Mannheim). The following method was recommended by S. Wanstall (per.comm. 1981) based on the methods described by Folch et al (1957) and Zollner and Kirsch (1962). Samples of 50 - 100 µg of liver were taken from the left lobe and where possible from a region clear of obvious infection. Samples were placed in 10 ml of 70% ethanol at $4^{\circ}C$ where they could be stored until homogenization, which was carried out for 5-10 minutes in an

ice bath. The homogenate was then centrifuged at 2000 r.p.m. for 10 minutes and the supernatent discarded. The tissue was resuspended in 1 ml. of three parts ether to one part 70% ethanol and spun again but this time the supernatent was retained. This tissue was resuspended in 1 ml. of the ether/ethanol mixture and spun, the supernatent being added to the previous sample. This was placed in a fume cupboard for evaporation until 0.1 - 0.05 ml. remained. The method outlined in the kit was then followed and the final solutions read in a CE 303 Grating Spectrophotometer against a standard at wavelength 530 nm. Lipid in mg/g liver was calculated from results using the formula below.

The standard contained 12.195 µg of lipid.

$$\frac{12.195}{A(\text{standard})} \times A(\text{sample}) \times 40 = \mu g \text{ of lipid in sample}$$

(40 = Dilutation factor)

$$= \frac{\mu g \text{ lipid}}{\text{mg liver tissue}} = \text{lipid mg/g liver}$$

PARASITE

Extraction of spores from fish tissue

One of three methods was used to remove microsporidian spores from the host tissue. Two depended on initial homogenization and the third on enzyme digestion, details are given below. In the first method the release of spores was facilitated by homogenization of tissue in 0.1M potassium hydroxide (Weidner 1972). This is based on the assumption that the resistant chitin-protein spore coat will not be affected by potassium hydroxide. An alternative method was to leave out the potassium hydroxide and

use Young's Teleost Saline only during homogenization. The third method was to use 5% trypsin in Young's Teleost Saline to digest the tissues around the spores. The first method proved most satisfactory, producing the cleanest spore suspension and was used in subsequent extractions. Following extraction, spores were washed three times by repeated centrifugation at 2000 r.p.m. in Young's Teleost Saline. They were stored at 4°C in Young's Teleost Saline or distilled water with 0.05 ml. Penicillin/Streptomycin to control bacterial growth. It was essential to know the condition of the spores after extraction procedures to ensure that a significant number survived. Spores were checked usually by the aid of phase contrast microscopy, particular attention being given to spore integrity. In addition samples were tested for filament extrusion, this being induced by exposure to the oxidizing agents saturated Iodine solution and diluted hydrogen peroxide. The percentage of extruded sporoplasms was taken as an indication of spore viability.

Administration of spores to fish

In an attempt to initiate experimental infection of microsporidia, spores were introduced to the fish host by one of two routes namely *per os* and intraperitoneal injection. Where anaesthetization was necessary the fish were placed in MS 222 and then held with tissue paper while spores were introduced. The fish were then placed in fresh aerated water to recover before returning them to the experimental aquaria. If necessary fish were labelled at this stage with coloured latex injected into the ventral flank. Where spores were required in suspension, extraction was carried

out by homogenization in Young's Teleost Saline as described earlier. Introduction of spores per os was undertaken either directly using a stomach tube (hyperdermic syringe with cannula tubing) or indirectly as food. Spores for direct per os introduction were administered in 0.5 ml. aliquots at a concentration of 1.28×10^7 spores per ml. of Young's Teleost Saline. Indirect introduction of spores as food to the experimental host was carried out by two methods. One, by feeding fish freshly extracted liver from Taurulus bubalis infected with microsporidia, and two, by introducing Daphnia or Artemia previously exposed to a concentrated spore suspension for an hour as food to the experimental host. Checks were made to ensure that each fish consumed the contaminated food. A further method of introducing spores to an experimental host was by intraperitoneal injection of 0.2 ml. spore suspension in Young's Teleost Saline, at the same concentration used by stomach tube administration. Anaesthetic was used for both these methods as described earlier.

HISTOLOGY

Material for sectioning was removed for fixation immediately on death of the host.

Light microscopy

Rapid methods

Smear preparations were used to confirm the presence of the parasite and to determine its stage of development. The area of tissue to be investigated was smeared onto a slide, allowed to dry and fixed for 2 minutes in methanol. It was then stained in the standard Giema stain (1 part

giemsa to 9 parts buffer) for 30 minutes. The slide was rinsed in buffer, allowed to air dry and immediately examined under oil on a Zeiss microscope. Tissue prepared for sectioning on a Bright Cryostat were frozen using liquid nitrogen and cut into 8 µm sections. Sections were picked up on slides ready for staining. For wax sections the tissue was fixed in either formal saline or Bouin's fixative for a minimum of 18 hours, depending on size. The material was dehydrated in alcohol and cleared in xylene, this process being carried out automatically on a Shandon Elliot automatic tissue processor. The tissue was then infiltrated by wax under vacuum before blocking up. Sections of 0.07 µm were cut on a Leitz Microtome. For JB-4 resin, material was fixed for four days in formal saline before dehydrating and embedding as explained in the manufacturer's instructions. Polymerization took three hours at room temperature and blocks were stored with silica gel to facilitate hardening of resin. Sections of 2 µm were cut on a Reichert Ultramicrotome.

Staining techniques

Cryostat sections were stained using the methylene blue technique for rapid biopsis (Pearse 1968). Mallory's Triple, Haemotoxylin and Eosin stains were used on wax sections. The yellow colouration of Bouin's fixative being removed prior to staining by lithium carbonate. Periodic Acid-Schiff with diastase control was carried out on wax sections according to Pearse (1968). Modified stains were used on JB-4 resin sections as advised by the manufactorers which included Heidenhains 'Azan' stain being used in place of Haemotoxylin and Eosin.

Electron microscopy

Scanning electron microscopy

Spores were separated from host tissue as previously described, and fixed for five days in 3% glutaraldehyde. The spores were then spun down and rinsed in buffer before post fixing in 1% osmium tetroxide for two hours. After rinsing by centrifugation again the spores were slowly dehydrated taking 12 hours to reach absolute alcohol. Spores were then dried in one of two ways, by air drying or critical point drying. For air drying a drop of suspension was placed on a stub and allowed to dry. Critical point drying required the spores to be placed in capsule with a millipore filter before placing in a Samdri pvt-3 critical point dryer. The filter was then placed on a stub for examination. The spores were coated with 16 nm of gold on a Polaron SEM Coating Unit E1500 before examining on a Joel JSM 35 Scanning Electron Microscope.

Transmission electron microscopy

Pieces of liver approximately $1mm^3$ were placed in 3% glutaraldehyde in O.IM sodium cocodylate buffer, pH 7.2, or formaldehyde glutaraldehyde solution (Karnovski 1965) for 8 hours at $4^{o}C$ and then rinsed. Post fixation with 1% osmium tetroxide was carried out before rinsing and dehydrating in ethanol prior to embedding in epoxy resin. Propylene oxide was used to facilitate the infiltration of resin. Spurr's epoxy resin (Spurr 1969) was found to be the most satisfactory embedding medium requiring curing for 8 hours at $70^{o}C$. Sections 0.05 µm - 0.07 µm were cut using a Reichert Ultramicrotome and collected on uncoated copper

grids. Sections were stained with a saturated solution of uranyl acetate for 10 minutes and for a further 10 minutes in lead citrate (Reynolds 1963) before examination on a Philips 300 transmission electron microscope at KV 60 and 80.

STATISTICAL ANALYSIS

Statistical analysis was concerned with the relationships between the intensity of infection with age and sex, and with the effect of infection on condition factor, liver weight, gonad weight and lipid content of the liver of the host. All data used in the analysis can be found in Appendix II. Computer analysis, including Chi-square tests were undertaken on a PDP/11/40 computer running in BASIC PLUS. Samples from Portwrinkle were analysed in detail whilst mean values of host variables and infection levels were compared between the 5 collection stations. It was not possible in the time available to obtain sufficient information from all sites for detailed comparisons.

Chi-square analysis was used because of the discontinuous nature of the data collected on infection levels. (Analysis of variance requires proportional or equal data which was not available). Ranges of host variables were compared to infection levels of both hepatic microsporidia and metacercaria. The program ALTER selected two columns from the file and on the command PICK they were divided up into quartiles (Table 2). TAB 2 sets the converted data into two way frequency tables for Chi-square tests to be carried out using the program CHSQ 2. This test indicated the dependance or independance between infection and each host variable. A significance of ninety five per cent ($P < .05$)

Table 2 To show divisions of variables into quartiles for Chi-square analysis
(LQ - Lower quartiles UQ - Upper quartiles)

HOST/PARASITE VARIABLES	MINIMUM - LQ	LQ - MEDIAN	MEDIAN - UQ	UQ - MAXIMUM
LENGTH cm	0 - 4.59	4.6 - 5.99	6.0 - 7.59	7.6 +
WEIGHT gm	0 - 2.49	2.5 - 5.49	5.5 - 9.99	10 - 100
CONDITION FACTOR	0 - 2.29	2.3 - 2.66	2.67 - 2.89	2.9 - 100
AGE yrs.	0	1	2	-
SEX	1	2	-	-
GONAD WEIGHT mg.	0 - 1.99	2.0 - 14.99	15 - 699	700 - 1000
GONAD WEIGHT % BODY	0 - 0.029	0.03 - 0.269	0.27 - 0.549	0.55- 10
LIVER WEIGHT mg.	0 - 49.9	50 - 99.9	100 - 179.9	180 - 1000
LIVER WEIGHT % BODY WEIGHT	0 - 1.649	1.65 - 2.09	2.1 - 2.69	2.7 - 100
TOTAL LIPID LIVER WEIGHT mg	0 - 0.99	1.0 - 2.99	3.0 - 7.99	8.0 - 100
LIPID % LIVER WEIGHT	0 - 0.249	0.25 - 0.49	0.5 - 0.99	1.0 - 10
MICROSPORIDIAN INFECTION	0	1	2	3
METACERCARIAL INFECTION	0	1	2	3

or more was taken as a rejection of the null hypothesis which states that there was no dependance between the variables.

Mean values of each variable with standard error of mean were plotted for each site to compare populations. Student's t-tests were used to determine the significant difference between the means.

Age/sex combinations were investigated to see if they had an effect on the level of microsporidia and metacercaria infection levels. A ninety five per cent confidence interval was used for each value, the following (non-parametric) method was used to calculate these, (R. Clark pers. comm.1982).

Let Xi = number of 1's in age/sex combination

Let Pi = proportion of 1's in age/sex combination

Let Ni = total sample number

I = infection present

The maximum likelihood of estimator Pi $= \frac{Xi}{Ni}$

Variable mean (pi) $= \frac{1}{N^2}$ Ni Pi (1-Pi) $=$ Pi $\frac{(1-Pi)}{Ni}$

Therefore 95% Confidence Interval for Pi is approx.

Other plots comparing host variables over time have included standard errors as large variations do occur and an indication of such variation is essential when drawing conclusions from the average plot.

RESULTS

Results are treated under three main sections, a description of the parasites, experimentation and host parasite relations.

MICROSPORIDIA - DESCRIPTION OF PARASITES FROM 6 DIFFERENT HOST SPECIES

Microsporidia were investigated from the liver of 5 species of fish namely Taurulus bubalis, Gaidropsarus mediterraneus Ciliata mustela, Crenilabrus melops and Scopthalmus maximus. In order to investigate tissue specificity, studies were also made on microsporidia from the musculature of one of the above mentioned fish, namely T.bubalis and a closely related species from the freshwater habitat Cottus gobio. Species from the muscle tissue of S.maximus were identified as Tetramicra brevifilum.

Hepatic microsporidia from Taurulus bubalis

In this host all stages in the life cycle of the microsporidian occurred within host liver cells here thought to be hepatocytes. Intracellular development of the parasite is considered under merogony, sporogony and spore formation and from these studies the species was found to fall within the genus Microgemma (Ralphs 1985).

Merogony

Stages identified as merogonic plasmodia (Figs. 9,10,11,12, 13) were generally multinucleate and surrounded by a vacuole bounded by a cell membrane continuous with the host cysternae of endoplasmic reticulum (Fig.14). The contours of the meront are closely followed by this host cell

membrane leaving a vacuolar space of 0.03 μm - 0.06 μm. Merogonial stages varied in size and shape from small spherical plasmodia measuring 3 μm to large elongated plasmodia of 18 μm. The cytoplasm consists of scattered ribosomes, parallel rays of endoplasmic reticulum and vesicles bounded by a double membrane of 0.008 μm - 0.0130 μm. What appeared to be pinocytic vesicles were identified in figure 13. The number of nuclei varied from a maximum of twenty observed in an elongated plasmodia to less than five in small spherical ones. Occasionally small spherical cells were located with a single nucleus (fig. 43) although this could not be confirmed from a single section. The nuclei (Figs.15, 16) are roughly spherical and measure between 1.1 μm and 1.7 μm in diameter with a prominent nucleolus. The nuclear membrane, again a double membrane system of 0.01 μm is continuous with the endoplasmic reticulum of the cytoplasm and interrupted by pores of approximately 0.0475 μm. The division of the nucleus precedes that of the cytoplasm and is characterized by depressions in the nuclear envelope (fig.16) which are associated with the spindle plaque. Double membraned polar vesicles are clearly associated with spindle plaques during division. At no stage in the life cycle were the nuclei observed in the diplokaryon state. After cytokinesis the surrounding host membrane bound vacuole appears to break and rejoin around each newly formed meront.

Sporogony

Entry of meronts into the sporogonic phase of development is marked by the absence of a surrounding membrane of host origin, the parasite lying free in the cytoplasm, and the

deposition of extramembraneous material. The plasmodia (fig.17) found free in the host cytoplasm without a host membrane bound vacuole or extramembraneous deposit appears therefore to represent a transitional stage between the meront and the sporont. Whether this stage undergoes nuclear division is not known although characteristic sporont stages with up to five nuclei suggests that they do.

The sporonts (figs.18,19,20,21,23,24) measuring between 4 µm - 8 µm have two to five nuclei and a similar cytoplasmic composition although slightly more vesicular to that of the meront. Extramembraneous material is deposited at irregular intervals in units of approximately 0.003 µm (figs.18,20). Although nuclear division was rarely observed in the sporont, cytoplasmic division was a regular feature and occurred by both single budding (fig.18) and rosette formation of 3 - 5 buds (figs.21,22). This resulted in an indeterminate number of mother sporoblasts being produced by one sporont. The second phase represents the nuclear and cytoplasmic division of the mother sporoblast cells into two rounded sporoblasts measuring approximately 3.28 µm by 2.4 µm (figs.25,26). The cytoplasm at this stage is highly vacuolated with a well developed membrane system. The deposition of extramembraneous material is completed by this stage.

Spore

It is convenient to describe the structure of the spore before considering sporoblastic development. The spore is oval in shape, narrowing slightly towards the anterior end, measuring 2.6 µm by 1.2 µm from electron micrographs.

(measurements varying considerably from fresh preparations). The spore wall is clearly defined and divided into two layers external to the cell membrane (fig.27), the exospore measuring 0.0026 μm and the electron translucent endospore 0.0051 μm. The endospore becomes narrower at the anterior end of the spore (fig.28) where the anchor disc meets the spore wall. The polar filament originating from the posterior end of the spore passes forward to the anchor disc, forming seven to ten coils around the posterior vacuole which are occasionally arranged in double rows (fig.27). The filament is approximately 0.0091 μm in diameter and consists of concentric layers. The extruded filament (fig.29) has an irregular surface and a groove visible along its length. The point of attachment to the spore wall appears to be the everted anchor disc (figs.27, 31,32) which is closely contoured by a membrane continuous with the lamella of the polaroplast. The lamellae is a complex series of radially arranged membranes (fig.27) which are in turn continuous with the endoplasmic reticulum. In addition to the lamellae the polaroplast consists of a vesicular region (fig.28) which is less pronounced in older spores. The posterior vacuole bounded by a unit membrane occupies approximately one third of the spore volume and may contain inclusion bodies (fig.27) and other particulate matter. The sporoplasm consists of a single nucleus and cytoplasm dense in ribosomes and membranes. Scanning electron micrographs show the spore coat to consist of an irregular pattern of small 'lumps' (fig.33) and the indentation adjacent to the collapsed posterior vacuole is indicative of a tubule (fig.34).

Sporoblast

By this stage the extramembraneous deposit is completed and during spore development forms two distinct layers, the exospore and later the endospore. The highly vacuolated cytoplasm begins to synthesise spore organelle. The cysternae of membranes at the posterior end (fig.36) may be regarded as golgi apparatus. The first recognisable spore organelle are the rudiments of the polar filament (fig.35). This begins in the posterior region of the spore and its close association with the golgi apparatus can be seen. Except for the two outermost rings the concentric rings of the polar filament gradually disappear during spore development. During filament formation the posterior vacuole becomes visible being relatively small at first taking up one eigth of the spore volume, it increases to approximately one third. Occasionally an electron dense body was seen in immature spores (fig.37) preceding the posterior vacuole. However this did not resemble the inclusion body in figure 27. The polaroplast is also being synthesised at this time developing from sac like vesicles known as the vesicular polaroplast (fig.38).

Xenoma

Microgemma sp. induces host cell hypertrophy and reorganization of contained organelle. These xenomas measured between 35 μm and 750 μm, 4 - 20 times larger than the adjacent hepatocytes. The smallest xenomas (figs.39,40) were investigated only at light microscope level. Spores were located even in this xenoma indicating that both merogony and sporogony are continuous processes throughout the infection. The ultrastructural organisation of the

surface layers of the xenoma showed characteristic features indicative of intensive metabolic activity at the cell surface such as microvilli (fig.41) and junctional complexes (fig.42). However the mitochondria within the xenoma are different to those in the normal host cells being more translucent, one and a half times larger and with disorganized cristae. The outer peripheral zone, (fig.43) is devoid of parasitic stages but has dense host cytoplasm consisting of ribosomes, glycogen, endoplasmic reticulum and abundant mitochondria. The next distinct zone is recognised by reticulated host nuclei interspersed with mitochondria (fig.44). Upon invasion of the cell the host nucleus begins to enlarge and lose its spherical shape (fig.45) then it appears to fragment and is later pushed to the periphery of the cell. The inner zone is comprised of parasitic stages and can be loosely divided into three regions. The outermost region (fig.44) consists mainly of meronts with a few scattered spores and abundant mitochondria which are closely associated with the developing stages. The next region is occupied by sporonts (fig.46) with the occasional meront and spore. Spores and sporoblasts are encountered in the central region of the xenoma (fig.47) and increase in numbers with the maturation of the xenoma. When spore production is completed the host cell cytoplasm disintegrates leaving a mass of spores (fig.48). Host reaction changes with development of the xenoma (figs.49,50,51,52). In mature xenomas where all parasitic development has ceased fibroblast cells begin to appear at the cell surfaces (fig.49) which is indicative of a granuloma reaction to the parasite. In old infections the entire complex is encapsulated by the deposition of collagen (fig.50,56).

In these cells phagocytes are associated with the degeneration and breakdown of the xenoma and later with ingestion of spores (figs.52,53,54). It is these later stages of the xenoma that are most clearly seen at light microscope level as white foci of infection (fig.8). Figures 55 and 56 show further details of parasite distribution throughout the liver. Staining with periodic acid - Schiff shows the liver to be rich in glycogen despite the high levels of parasitic infection. The xenomas however do not appear to contain polysaccharides although the hemiuridae do. Mallory's Trichrome indicates areas of connective tissue which stain blue around the hepatic vessels and xenomas. Concentration of dark red stain in the xenoma shows a high proportion of nuclei material.

Comparison of spores of hepatic microsporidia from *Gaidropsarus mediterraneus*, *Ciliata mustela* *Crenilabrus melops* and *Scopthalmus maximus*

One of the aims of this study was to determine the host specificity of the agent or agents involved in hepatic microsporidiosis. Such an assessment would ideally be based on comparative studies of all stages of parasitic development and on the cross infectivity of spores. Unfortunately here, due to limited time, only chronic infections were found in all species permitting ultrastructural and morphometric studies of the spore alone to be undertaken. Furthermore the intensity and incidence of hepatic infection (Table 3) in the above species was far lower than that of *T.bubalis*.

Morphometric data is also listed in this table from which

TABLE 3 To compare spore characteristics of nine Microsporidia from seven species of fish host

Hepatic Microsporidian sp. of	Incidence of Infection (% fish infected)	Spore dimensions length µm	Spore dimensions breadth µm	Condition of spore	No. of coils	Length of extruded filament µm	Nuclear condition of spore
Taurulus bubalis	92%	4.6 ± 0.47 2.453	2.3 ± 0.21 1.43	Fresh Fixed	7-10	53.7 ± 3.8	Uninucleate
Gaidropsarus mediterraneus	57%	4.5 ± 0.51 2.390	2.5 ± 0.41 1.45	Fresh Fixed	7-8		Uninucleate
Ciliata mustela	42%	4.244 ± 0.38 2.67 ± 0.25	2.043±0.22 1.244±0.13	Fresh Fixed	7-8	43.11±3.54	Uninucleate
Crenilabrus melops	46%	4.5 ± 0.37 2.45 ± 0.27	2.3 ± 0.36 1.4 ± 0.178	Fresh Fixed	7		Uninucleate
Scopthalmus maximus	-	2.51 ± 0.35	1.43 ± 0.15	Fresh Fixed	6-7		Uninucleate
Chelon labrosus	60%	4.3 ± 0.45 4.2	2.4 ± 0.36 2.4	Fresh Fixed	7-9	56.72±9.18	Uninucleate

TABLE 3 (Contd.) To compare spore characteristics of nine Microsporidia from seven species of fish host.

Intramuscular Microsporidian sp. of	Incidence of Infection (% fish infected)	Spore dimensions Length µm	Breadth µm	Condition of spore	No. of coils	Length of extruded filament	Nuclear condition of spore
Taurulus bubalis	32%	3.76 ± 0.406 2.42	2.12±0.156 1.33±0.148	Fresh Fixed	6-8		Uninucleate
Cottus gobio	6%	2.78 ± 0.25	1.2 ± 0.19	Fresh Fixed	15-17		Uninucleate
Scopthalmus maximus	-	4.8	2	Fresh Fixed	3-5	50 µm	Uninucleate

it can be seen that the measurements of fresh spores from the 5 hosts fall within the range of 4.2 µm - 4.6 µm long and 2.0 - 2.5 µm wide making it impossible to separate spores on this basis. Similarly there appeared to be no significant difference between the length of extruded polar filaments measuring approximately 45 - 55 µm.

Ultrastructural investigations (fig.57) indicate an overall similarity in spore organization between all hepatic spores investigated here and although these represent different stages of integrity due to host reactions the characteristics fall within those found in T.bubalis. This includes the 7 - 8 coils of polar filament, a posterior vacuole and the presence of an inclusion body. The spore from C.melops however exhibited minor differences such as a smaller posterior vacuole and a more prominent nuclear membrane than the other hepatic spores investigated here. The use Karnovski's fixation methods (Karnovski 1965) in the preparation of this material may be responsible for these variations. Overall there is no evidence from the spore studies to suggest that separate species are involved. However as previously mentioned further studies on developmental stages are necessary before definite conclusions can be drawn.

Microsporidia from the musculature of Taurulus bubalis and Cottus gobio

Studies were carried out on microsporidian infections from sites other than the liver in T.bubalis in an attempt to investigate tissue specificity. In addition studies were made of a microsporidian from the musculature of G.gobio

a freshwater species closely related to T.bubalis in the family Cottidae. The screening of C.gobio for microsporidiosis was undertaken as it was intended for this fish to be used as an experimental host for the elucidation of the life cycle of Microgemma sp. It was however only necessary to determine whether these intramuscular muscle microsporidia resembled the hepatic species and so detailed studies were not envisaged. Nevertheless it was possible to confirm that two species were involved, Pleistophora vermiformis (Léger 1904) in C.gobio and Pleistophora sp. in T.bubalis and as there have been no previous ultrastructural studies, brief descriptions are given. The pansporoblastic nature of both species clearly distinguishes them from the hepatic microsporidia investigated here (fig.58, 59).

Pleistophora sp. from Taurulus bubalis

Parasitic stages were found in elongated masses in the internal body wall musculature. The merogonic stages varied in size from less than 3 µm to 10 µm (fig.60) and showed prominent nuclei. Sporonts (fig.63) were characterized by extramembraneous deposits and measured approximately 5 µm. Microtubules were visible in the parasitiphorous cytoplasm and the close proximity of the host mitochondria to the parasitiphorous vacuoles were noted (fig.65). Characteristic features of the spore were its dimensions 3.76 µm by 2.12 µm from fixed samples and 6 - 8 coils of polar filament (fig.66). Figure 61 shows details of the anterior end of the polar filament with its lamella polaroplast.

Pleistophora vermiformis from Cottus gobio

Parasitic stages were recorded externally from the posterior

ventral musculature. Sporogonic plasmodia measuring approximately 5 μm were again distinguishable from the meronts by their extramembraneous deposits (fig.62). Spore dimensions of 3.7 μm by 2.1 μm were recorded from fixed samples together with 15-17 coils of polar filament (fig. 67). Fixation procedures used for hepatic microsporidia did not give as good results with intramuscular species of Pleistophora. The late sporoblasts appeared stellate (fig.64) and the spores distorted. Similar fixation difficulties were encountered by other workers in this field, notably Canning et al (1979) and Lom and Corliss (1967).

ECOLOGICAL ASPECTS OF TAURULUS BUBALIS

Taurulus bubalis was generally found to inhabit reasonably sheltered rocky shores such as Portwrinkle, Wembury, Roscoff and Aberystwyth but it also occurred on the exposed rocky shores of Widemouth and Newquay (Table 1). It did not however appear to colonize very exposed shores such as Portleven in south-west Cornwall (fig.2). Fish were collected throughout the year at Portwrinkle and Wembury from pools between mid-tide level and low water springs. Fish greater than 6cm were generally found lower down the shore than immature juveniles, particularly in the spring when spawning takes place and sexually mature fish migrate to the low water zone. Immature males of T.bubalis, found at the mid-tide level in spring exhibited high levels of infection with hepatic hemiurid metacercaria. In late spring and early summer juvenile T.bubalis were found in sandy bottom pools all over the shore, but were particularly concentrated in pools connected to the sea. The number of '0' group fish decreased towards October which

was attributed to the high mortality of juvenile stock.

T.bubalis is a voracious predator, taking chiefly crustacea but sometimes fish. Observations in the aquaria also showed that they were not adverse to cannibalism. On inspection of the stomach contents of juvenile fish the crustacean Dynamene bidentata and the malacostracan Nebalia bipes were found. Stomachs of adult fish contained primarily crustacea, including small crabs, shrimps and isopods, namely Idotea baltica. The method of feeding employed by T.bubalis depends on concealment, lying in wait for passing prey on which it pounces. The concealment is enhanced by the use of camouflage, fish living in Fucus sp. take on an olive hue, whilst those amongst red algae such as Corallina officinalis are reddish. By maintaining periods of inactivity while waiting for prey and hiding in crevices, capture is made difficult even when using the anaesthetic quinaldine. The sexually mature male during the breeding season has a deep yellow ventral surface with contrasting black markings around the base of the pectoral fins. Females do not take on breeding colouration but were noticeable by their swollen abdomens when ready to spawn. The territorial behaviour of T.bubalis is marked and becomes more effective on maturity. This limits the number of fish to between three and five per $1.5m^3$ rockpool, however young recruits when they first appear on shore in June and July are found in densities of 20 - 30 per $1.5m^3$ rockpool.

Associated species in the rockpool environment

Studies indicate that T.bubalis co-exist with several fish species although their degree of interaction is unknown.

Ciliata mustela and Gaidropsarus mediterraneus occur in similar densities to T.bubalis whereas Crenilabrus melops and Gobius niger are slightly more abundant. Lipophyrs pholis however occurs at densities 3 - 4 times greater than T.bubalis.

Populations do vary with season and the recruitment of juvenile fish will increase populations accordingly. Gobisculus flavescens and juvenile Callionymus lyra were not encountered on every sample trip but were recorded on the majority of occasions. Crustacea within the pool environment include Carcinus maenas, Cancer pagurus, Crangon vulgaris, Gammarus locusta, Macropipus puber, Eupagurus prideaux, Leander squilla, Nebalia bipes, Idotea baltica, Jassa fulcata and Balanus balanoides, many of which fall prey to the forementioned species.

EXPERIMENTAL INVESTIGATIONS OF TRANSMISSION

Before attempts could be made to infect experimental hosts it was necessary to evaluate spore viability following extraction and storage. Two experiments were undertaken, one to investigate the optimum oxidizing agent for testing sporoplasm extrusion - the main criteria used here to test spore viability, and one to determine the effects of storage. These were followed by 5 experiments which were designed to study infection. Spores used in all experiments were extracted from the liver of Taurulus bubalis from S.W. Britain by homogenization in potassium hydroxide as described earlier in Materials and Methods.

Experiment 1: To investigate the effect of the oxidizing agents saturated iodine water and hydrogen peroxide on filament extrusion of Microgemma sp. from Taurulus bubalis

Although saturated iodine water is known to bring about filament extrusion it was necessary to confirm its effect on Microgemma sp. from Taurulus bubalis, the activity of which was compared with different concentrations of hydrogen peroxide. The spores were extracted from several fish caught in April when ecological studies indicated infection to be in the chronic phase and pooled for use in this and the following experiment. Spores were stored in Young's Teleost Saline for use 2 hours later. The experimental procedure required addition of two drops of the appropriate oxidizing agent to one drop of spore suspension on a glass slide under a coverslip. This was carried out at room temperature. Drops were dispensed in approximately equal volumes and therefore considered equal to a dilution factor of 2 : 1. Preparations were examined at 5 and 30 minute intervals by counting approximately 30 spores within each of 10 fields of view at 1000 X magnification. Results are tabulated below.

Concentration of Hydrogen Peroxide Solution		Percentage exsporolation after	
		5 mins.	30 mins.
'Stock'	After 2 : 1 dilution		
6 %	4.0%	5	15
5.4%	3.6%	16	39
4.8%	3.3%	20	70
4.2%	2.8%	12	30
3.6%	2.6%	3	4
2.4%	1.6%	0	0
Saturated Iodine Water		20	64

The results indicate relatively small variations in concentrations of hydrogen peroxide leading to marked differences in percentage exsporulation. High concentrations of greater than 3.6% appear to have a suppressive effect on exsporolation while low concentrations of less than 2.8% were insufficient to stimulate filament extrusion. Following this experiment a final concentration of approximately 3.3% was used to test viability prior to other studies. Although this was effective in stimulating filament extrusion in the majority of instances there were occasions, notably in winter, when spores failed to respond even when experimental conditions were maintained as above.

Experiment 2: To investigate the survival of spores maintained at 4°C with time

Spores investigated in this study were obtained from the same pool as those used in Experiment 1. Following extraction they were stored in Young's Teleost Saline with 0.05ml penicillin/streptomycin per cm^3 of spore suspension at 4°C in air tight sterile containers in the dark. Filament extrusion was taken to indicate survival following exposure to saturated iodine water of hydrogen peroxide. Tests were carried out on spores after 7 days and 28 days and compared to percentage exsporulation achieved on day 1. Results are shown below.

Time interval following extraction from host	% Filament Extrusion after 10 minutes	
	H_2O_2 (Stock 4.8%)	I_2
Day 1	16	20
Day 8	32	13
Day 28	30	20

There was no evidence from the results overall to show a fall off in inability to exsporulate during the period investigated.

Experiment 3: To investigate infection of Taurulus bubalis maintained at 15°C with spores of Microgemma sp. introduced per os

A total of 12 fish between 4cm and 6.5cm in length were used in this experiment, 6 as controls and 6 as experimental hosts. Fish from Aberystwyth were selected because of their naturally low levels of infection, there being no site investigated found to be totally free of microsporidiosis. Control and experimental fish were maintained in separate tanks at 15°C. Spores were extracted from fish caught in September, by homogenization with potassium hydroxide and stored overnight in Young's Teleost Saline at 4°C, before administration to the experimental hosts via a stomach tube as described in Materials and Methods. Fish were examined at six and eight weeks, half the fish killed at the first interval and the remainder at eight weeks. Livers were examined for microsporidia by fresh or stained squash preparations. In addition fresh frozen sections were cut at random and at suspect areas within the liver for closer examination. There was no evidence found for new infections from the introduction of spores. Neither the controls nor the experimental fish on examination had established infections of microsporidia which might have resulted from natural infection before the experiment.

Experiment 4: To investigate infection of post-larval Taurulus bubalis, using a variety of methods for the introduction of spores

40 fish measuring less than 3cm in length were collected

from Portwrinkle in early July. They were divided into 5 equal groups, namely A, B, C, D and E and maintained in separate tanks at 17°C. Spores were obtained from freshly killed T.bubalis collected at the same time, extracted by potassium hydroxide and stored in Young's Teleost Saline over night. Spores were introduced into each group as follows; group A by intraperitoneal injection, group B by stomach tube, group C by feeding directly on infected liver and group D by exposing the host to Artemia which had been previously exposed to a concentrated spore suspension. Group E was used as the control. Details of each method are described in Materials and Methods. Fish were sacrificed at 4 and 8 week intervals but no infection was found.

Scopthalmus maximus was chosen as an experimental host for two reasons, the first being that hepatic microsporidia are known to occur in specimens from Widemouth and the second being previous success with transmission of another species Tetramicra brevifilum (Matthews and Matthews 1981).

Experiment 5: To experimentally infect 'O' group Scopthalmus maximus with hepatic microsporidia

A total of 17 'O' group turbot were used, kept together in a large black polypropylene tank. Latex was used to mark the fish indicating whether they were control fish, infected with spores via stomach tube or via intraperitoneal injection. Spores were extracted by homogenization with potassium hydroxide and kept over night in Young's Teleost Saline at 4°C. Fish were sacrificed at 4. 6 and 8 week intervals. The results are tabulated overleaf.

Time of Sacrifice in weeks	Fish infected with Hepatic Microsporidia from T.bubalis				Control	
	via intraperitoneal injection		via stomach tube			
	presence of T	M	T	M	Presence of T	M
4	P -	O -	O	O	O O	O O
6	O P -	P P -	P O -	O O -	P P P	O O O
8	O P	P P	P P	P O	O P	O P

T = Tetramicra brevifilum
M = Microgemma sp.

The fish that had been administered spores via intraperitoneal injection had small white spots in and around the liver membranes. On close inspection these were found to be phagocytosed microsporidian spores. It was these that were recorded as Microgemma sp. present after intraperitoneal injection.

As with T.bubalis, S.maximus could not be guaranteed free of hepatic microsporidia. Cottus gobio however, being a close relative of T.bubalis and ecologically separated in the freshwater environment could be guaranteed free of hepatic microsporidia and was used in the following experiments.

Experiment 6: To experimentally infect Cottus Gobio with Microgemma sp. via intraperitoneal injection and per os

A total of 15 fish between 4cm and 6cm long collected in November were equally divided into 3 tanks maintained at

14°C. Spores were administered via stomach tube to one tank of fish via intraperitoneal injection to another, the third tank of fish acting as control. Fish were killed at 4 and 8 weeks and liver squashes undertaken. No liver infections were found, although a species of Pleistophora, not visible at the commencement of the experiment was discovered in the tail musculature of both control and experimental fish. Ultrastructural studies carried out on this species showed it to be distinct from that administered.

Experiment 7: To experimentally infect 'O' group Cottus gobio with Microgemma sp. using a variety of methods for the introduction of spores

This experiment was run simultaneously with Experiment 4, spores being obtained from the same source. 45 fish less than 3cm in length were collected from Shillamill Lakes Cornwall, in June. They were divided into 5 equal groups namely A, B, C, D and E and maintained in separate tanks at 17°C. Spores were introduced into each group as follows; group A by intraperitoneal injection, group B by stomach tube, group C by feeding directly on infected liver and group D by exposing the host to Daphnia which had been previously exposed to a concentrated spore suspension. Group E was used as a control. Details of each method are described in detail in Materials and Methods. Fish were killed and examined for hepatic infections at 4 and 8 weeks. No infection was detected.

ECOLOGICAL ASPECTS OF HOST-PARASITE RELATIONS

Host

A series of investigations were undertaken to determine the

seasonal variation of host variables, namely condition factor, gonad weight, liver weight and lipid content of the liver. These were considered relevant in the interpretation of pathological changes which might be attributed to the effect of the parasite. The studies were based on a total of 241 fish from Portwrinkle, of mixed age and sex with samples of approximately 20 fish per month. Prior to correlating this date with that of the parasite it was necessary to have a reliable method of ageing fish. Results of otolith ageing (fig.68,69) were compared to estimations taken from length frequency graphs (fig.70) in an attempt to devise a way of determining age without sacrifice. Since T.bubalis is a short lived fish, seasonal variation is important in estimating age by length. Good correlations were found between these methods for age groups 'O' and 'I' in spring and summer but age groups 'I' and 'II' became indistinguishable on the length frequency plot for autumn and winter. It was concluded that for accurate ageing the otolith technique was far superior.

The condition of the liver was estimated as liver percentage body weight and showed significant ($P<0.001$) variation with season (fig.71) according to Chi-square analysis. The mean value was at its lowest, 1.75% in May and July with the highest peaks of 3.3% occurring in June. Values rose during the autumn from 1.8% to 3.15% levelling out over the winter months at approximately 2.4%.

Condition factor also showed significant ($P<0.001$) variation with season (fig.72). The lowest mean value of 2.0 occurred in January rising to approximately 2.75 in spring before falling to 2.25 in summer. The highest peak of 3.23

was reached in September falling to 2.75 over the winter months.

In addition to liver percentage body weight, lipid content was investigated as lipid percentage liver weight (fig.73). In calculating this value it was assumed that lipid would be uniformly distributed throughout the organ and the study based on 87 fish of the original sample. The overall trend showed a rise in summer with a mean peak value of 2.18% in August followed by a decline over winter to a minimum of 0.1% in March. Values then rose to an August peak. This seasonal variation was again confirmed significant ($P<0.01$) by Chi-square analysis.

Investigations of the gonad included only weight measurements used as a possible indication of the potential fecundity of the fish. Values were again expressed as a percentage body weight. Initial investigations of values for both ovaries and testes produced a similar curve and have therefore been combined here. The results are shown in figure 74 and based on 176 of the 240 fish sampled. The lowest levels, less than 0.4% were recorded in the summer. A rise in mean gonad percentage body weight commencing in the autumn reached peaks of 1.75% in December and 2.375% in March before rapidly declining to 0.5% in April. The developmental state of the gonads were also recorded, gravid fish occurring from December through to March, with only spent and immature fish being found between late April and September. Male gonads became totally black on spawning but there was no evidence of immature females having black gonads. From data obtained here, spawning appears to occur mainly in March.

A comparison between populations of Taurulus bubalis collected from sites around south west Britain and Brittany

In order to investigate interpopulation variation of T.bubalis, mean values of host variables and infection levels were calculated for each season (Table 4) the site was sampled. Summer and autumn were chosen for comparative studies (Table 5) as these periods provided the most data. Sample sizes varied from 8 at Aberystwyth to 58 at Portwrinkle, and so it was necessary to use Student's t-test to determine the significance of differences shown between the populations (fig.75 a,b,c).

Results of the summer sampling show the T.bubalis population from Aberystwyth to be significantly longer with a mean length of 7.61cm than populations from Wembury and Portwrinkle which had mean lengths of 5.23cm and 5.1cm respectively (fig.75 a). The Widemouth fish population was also significantly longer than that from Portwrinkle. Autumn samples from Portwrinkle and Roscoff were the only populations distinguishable by their significantly different mean lengths. Although from figure 75 a, both summer and autumn populations appeared to have different mean weights, Student's t-test indicated that these were not significant.

The Aberystwyth population from the summer collections had a mean condition factor of 2.99, significantly higher than that of Widemouth and Wembury, while the autumn sampling showed Widemouth to have a significantly lower mean condition factor of 1.92, than the other samples. The Roscoff sample was also found to have significantly lower mean condition factor than Wembury or Widemouth.

Table 4 To show mean values and standard error of mean for T.bubalis variables from Aberystwyth, Widemouth, Wembury, Portwrinkle and Roscoff. (M=Mean) (SE=Standard error of Mean)

	Host Variables	Aberystwyth		Widemouth		Portwrinkle		Wembury		Roscoff	
		M	SE	M	SE	M	SE	M	SE	M	SE
(Spring)	Length (cm)					6.894	0.200	6.877	0.730		
	Weight (grm)					8.900	0.846	8.433	1.895		
	C.F					2.640	0.071	2.414	0.188		
	Age (yrs)					0.958	0.073	0.888	0.309		
	Sex					1.565	0.060	1.625	0.183		
	Gonad % BW.					0.920	0.309	1.390	0.781		
	Liver % BW.					2.121	0.098	2.166	0.282		
	Lipid % LW.					0.462	0.075	-	-		
	Met.					1.330	0.098	1.110	0.309		
	Mic.					1.902	0.095	2.000	0.333		
(Summer)	Length (cm)	7.612	0.661	6.560	1.048	5.229	0.277	5.065	0.541		
	Weight (grm)	14.900	3.273	11.330	4.938	5.515	1.015	5.432	1.357		
	C.F.	2.990	0.093	2.484	0.165	2.518	0.066	2.480	0.139		
	Age (yrs)	1.125	0.226	1.000	0.246	0.328	0.712	0.500	0.154		
	Sex	1.714	0.184	1.625	0.183	1.743	0.075	1.750	0.164		
	Gonad % BW.	0.224	0.083	0.243	0.123	0.226	0.042	0.090	0.051		
	Liver % BW.	1.714	0.179	1.728	0.134	2.483	0.147	1.842	0.241		
	Lipid % LW.	5.638	1.443	0.356	0.063	1.861	0.627	-	-		
	Met.	0.250	0.249	0.166	0.112	1.344	0.131	1.300	0.230		
	Mic.	0	0	0.916	0.287	1.707	0.137	1.350	0.254		

Table 4 (Cont'd)

	Host Variables	Aberystwyth		Widemouth		Portwrinkle		Wembury		Roscoff	
		M	SE	M	SE	M	SE	M	SE	M	SE
(Autumn)	Length (cm)			7.125	0.314	6.007	0.308	6.685	0.614	7.809	0.558
	Weight (grm)			7.100	1.718	7.483	1.012	9.928	3.560	15.719	3.785
	C.F.			1.920	0.431	2.800	0.076	2.840	0.204	2.500	0.121
	Age (yrs)			1	0	0.529	0.098	0.856	0.260	0.620	0.117
	Sex			-	-	1.477	0.076	1.429	0.202	1.600	0.128
	Gonad % BW.			-	-	0.305	0.047	0.917	0.250	0.403	0.071
	Liver % BW.			-	-	1.920	0.100	2.022	0.239	1.049	0.247
	Lipid % LW.			-	-	0.843	0.152	-	-	6.650	2.133
	Met.			0.750	0.250	1.627	0.144	1.428	0.368	0.524	0.163
	Mic.			2.250	0.479	1.942	0.141	2.425	0.404	0.857	0.198
(Winter)	Length (cm)	6.687	0.673			6.398	0.269				
	Weight (grm)	12.022	3.164			8.806	1.290				
	C.F.	3.467	0.164			2.539	0.082				
	Age (yrs)	0.750	0.313			0.410	0.088				
	Sex	1.571	0.202			1.596	0.068				
	Gonad % BW.	1.529	0.813			1.228	0.328				
	Liver % BW.	3.848	1.315			2.403	0.216				
	Lipid % LW.	-	-			0.680	0.168				
	Met.	0.142	0.134			1.930	0.122				
	Mic.	0.250	0.164			1.750	0.125				

Table 5 To show results of t-tests carried out on host data obtained from population of T.bubalis collected from different sites

A significant t-value ($P < 0.05$) indicates that the populations are different, rejecting the null hypothesis which states that the populations are the same.

Host Variable	Sites Compared (Summer)	t-Value	Degrees of Freedom	Significance $P < 0.05$
Length	Aberystwyth, Portwrinkle	3.8559	64	✓
	Aberystwyth, Widemouth	0.5362	18	-
	Aberystwyth, Wembury	2.547	27	✓
	Widemouth, Portwrinkle	2.256	68	✓
	Widemouth, Wembury	1.3512	31	-
	Portwrinkle, Wembury	0.1643	77	-
Weight	Aberystwyth, Portwrinkle	1.1	64	-
	Aberystwyth, Wembury	1.108	27	-
	Widemouth, Portwrinkle	0.59	68	-
	Widemouth, Wembury	0.43	31	-
Condition factor	Aberystwyth, Portwrinkle	14.16	64	✓
	Aberystwyth, Wembury	9.75	27	✓
	Aberystwyth, Widemouth	10.74	18	✓
	Portwrinkle, Wembury	1.90	77	-
	Portwrinkle, Widemouth	1.42	65	-

Table 5 (Cont'd)

Host Variable	Sites Compared (Summer)	t-Value	Degrees of Freedom	Significance P < 0.05
Age	Aberystwyth, Portwrinkle	18.26	64	✓
	Aberystwyth, Wembury	7.92	27	✓
	Widemouth, Portwrinkle	18.38	68	✓
	Widemouth, Wembury	5.329	31	✓
	Wembury, Portwrinkle	7.8	77	✓
	Aberystwyth, Widemouth	0.99	18	-
Sex	Widemouth, Aberystwyth	2.822	14	✓
	Widemouth, Portwrinkle	3.749	44	✓
	Widemouth, Wembury	3.17	27	✓
	Portwrinkle, Wembury	0.505	64	-
	Wembury, Aberystwyth	0.98	40	-
Gonad % Body weight	Aberystwyth, Wembury	15.82	16	✓
	Widemouth, Wembury	13.0	14	✓
	Portwrinkle, Wembury	22.12	42	✓
	Portwrinkle, Widemouth	1.229	36	-
Liver % Body weight	Portwrinkle, Aberystwyth	4.78	55	✓
	Portwrinkle, Widemouth	7.959	60	✓
	Portwrinkle, Wembury	5.768	59	✓
	Wembury, Aberystwyth	1.0967	16	-
	Wembury, Widemouth	1.5624	21	-
	Widemouth, Aberystwyth	0.25	17	-

Table 5 (Cont'd)

Host Variable	Sites Compared (Summer)	t-Value	Degrees of Freedom	Significance P < 0.05
Lipid % Liver weight	Aberystwyth, Widemouth	2.402	9	✓
	Aberystwyth, Portwrinkle	1.89	16	-
	Portwrinkle, Widemouth	1.71	17	-
Metacercarial infection	Portwrinkle, Wembury	0.646	76	-
	Portwrinkle, Aberystwyth	7.994	64	✓
	Portwrinkle, Widemouth	13.3	68	✓
	Wembury, Aberystwyth	6.568	26	✓
	Wembury, Widemouth	11.73	30	✓
	Aberystwyth, Widemouth	1.405	18	-
Microsporidian infection	Aberystwyth, Widemouth	5.339	18	✓
	Aberystwyth, Portwrinkle	13.7	64	✓
	Aberystwyth, Wembury	9.215	23	✓
	Widemouth, Portwrinkle	7.327	68	✓
	Widemouth, Wembury	2.79	31	✓
	Portwrinkle, Wembury	2.82	77	✓

Table 5 (Cont'd)

Host Variable	Sites Compared (Autumn)	t-Value	Degrees of Freedom	Significance P< 0.05
Length	Roscoff, Wembury	1.0462	26	-
	Roscoff, Portwrinkle	5.029	70	✓
	Roscoff, Widemouth	0.217	23	-
	Widemouth, Portwrinkle	0.902	54	-
	Portwrinkle, Wembury	0.905	56	-
Weight	Roscoff, Portwrinkle	0.99	70	-
	Roscoff, Widemouth	0.10	23	-
	Roscoff, Wembury	0.109	26	-
Condition factor	Widemouth, Roscoff	7.356	23	✓
	Widemouth, Portwrinkle	10.1713	53	✓
	Widemouth, Wembury	5.29	9	✓
	Roscoff, Portwrinkle	14.7	70	✓
	Roscoff, Wembury	5.821	26	✓
	Wembury, Portwrinkle	0.835	56	-
Age	Widemouth, Portwrinkle	4.208	53	✓
	Widemouth, Wembury	6.882	9	✓
	Widemouth, Roscoff	7.23	24	✓
	Portwrinkle, Wembury	4.098	56	✓
	Portwrinkle, Roscoff	3.0603	71	✓
	Wembury, Roscoff	3.36	27	✓

Table 5 (Cont'd)

Host Variable	Sites Compared (Autumn)	t-Value	Degrees of Freedom	Significance P < 0.05
Sex	Portwrinkle, Wembury	1.16	56	-
	Portwrinkle, Roscoff	9.287	69	✓
	Wembury, Roscoff	3.74	26	✓
Gonad % body weight	Portwrinkle, Wembury	0.26	39	✓
	Portwrinkle, Roscoff	7.212	43	✓
	Wembury, Roscoff	11.016	16	✓
Liver % body weight	Roscoff, Wembury	9.75	24	✓
	Roscoff, Portwrinkle	18.5	40	✓
	Portwrinkle, Wembury	1.613	39	-
Lipid % Liver weight	Roscoff, Portwrinkle	0.839	28	-
Metacercarial infection	Widemouth, Portwrinkle	3.154	53	✓
	Widemouth, Wembury	2.40	9	-
	Widemouth, Roscoff	1.458	23	-
	Portwrinkle, Wembury	1.150	56	-
	Portwrinkle, Roscoff	17.63	70	✓
	Wembury, Roscoff	5.96	26	✓

Table 5 (Cont'd)

Host Variable	Sites Compared (Autumn)	t-Value	Degrees of Freedom	Significance P< 0.05
Microsporidian infection	Widemouth, Portwrinkle	1.110	53	-
	Widemouth, Wembury	.256	9	-
	Widemouth, Roscoff	5.51	23	✓
	Portwrinkle, Wembury	1.176	56	-
	Portwrinkle, Roscoff	16.41	70	✓
	Wembury, Roscoff	7.99	20	✓

Summer sampling showed both Wembury and Portwrinkle populations to be significantly younger than those of Aberystwyth and Widemouth, and Portwrinkle to be younger than Wembury. In the autumn all populations sampled had significantly different mean ages, Widemouth supporting the oldest population with a mean age of 1 year and Portwrinkle the youngest with a mean age of 0.52 years.

The summer population at Widemouth (fig.75 b) was significantly less female dominated than that of Aberystwyth, Portwrinkle and Wembury, although all sites were shown to have more females than males. In the autumn however, Roscoff had a more female dominated population than Wembury or Portwrinkle.

Gonad percentage body weight shows the Wembury population in summer to have a significantly lower value than Widemouth, Aberystwyth and Portwrinkle, while in autumn it was shown to have a significantly higher mean gonad value than the other populations.

Portwrinkle (fig.75) has a significantly higher mean liver percentage body weight in summer than Aberystwyth, Widemouth and Wembury. In autumn however, Roscoff has a significantly higher value than Wembury and Portwrinkle. The lipid percentage liver weight of the Aberystwyth population was significantly higher at 5.64% than Widemouth but not Portwrinkle. Only Roscoff and Portwrinkle populations were comparable in the autumn and these did not show significant variation in lipid percentage liver weight.

Infection levels of Microgemma sp. and hemiurid metacercaria were compared between populations sampled (fig.75).

Infection with Microgemma sp. varied significantly between all populations with Aberystwyth supporting the lowest mean infection level of 1.707. In the autumn Roscoff had the lowest mean infection level at 0.857 of the population sampled.

During the summer Portwrinkle and Wembury populations of T.bubalis supported significantly higher levels of hemiurid metacercaria than Aberystwyth and Widemouth and in autumn they again showed higher levels of infection than Widemouth and Roscoff.

The overall percentage of infection with Microgemma sp. for each population is as follows. The figures result from the total sample number collected from that site (Appendix I). Aberystwith 17%, Widemouth 69%, Portwrinkle 90%, Wembury 81% and Roscoff 57%.

Distribution of hepatic infection of Microgemma sp. and the digenean hemiurid metacercaria within the Portwrinkle population of T.bubalis

A total of 241 fish of mixed age and sex, collected over a 30 month period between January 1981 and June 1983 were examined for hepatic infections with Microgemma sp. and hemiurid metacercaria. In order to look at monthly incidence levels for each parasite, fish from consecutive years were pooled into appropriate months providing a representative sample for each month. From figures 76 and 77 it can be seen that a greater percentage of fish were infected over the winter months than the summer, both parasites showing similar patterns of prevalence. Levels of fish infected with Microgemma sp. did not fall below 75% which was attained in June, 100% infection being recorded in

March, April, September and November. Prevalence of metacercarial infections were lower overall than those of Microgemma sp. minimum levels of 65% being recorded in June and 100% infection only recorded once in November.

While providing useful information, prevalence values shown in figures 76 and 77 only record the presence and absence of infection. Figures 78 and 79 however show the varying proportions of infection categories, namely absent, low, medium and heavy making up the incidence levels. This not only shows the predominant level of infection for each month but also indicates in the case of Microgemma sp. the progress of infection within the population throughout the year. Low levels of Microgemma sp. infection (less than 20 xenomas) occurred in all monthly samples except September. Medium levels of infection (20 - 70 xenomas) were present in all monthly samples and represented the most common level of infection (Table 4). Heavy infection levels (greater than 70 xenomas) were most abundant in March, April, August, September and November which coincided with small percentages absent and low infections. Unlike the pattern of microsporidian infection, low infection levels (less than 10 cysts) of hemiurid metacercaria were found in a remarkably constant percentage of fish between March and December. Medium levels of infection (10 - 30 cysts) peaked in February, September and December. Heavy infection levels (greater than 30 cysts) were found in few fish particularly over the spring and summer and were absent in July and September.

TABLE 6: To show overall percentage of each infection category for both Microgemma sp. and hemiurid metacercaria

Infection Category	Microgemma sp.	Hemiurid Metacercaria
Absent	10.8	17.2
Low	26.8	28.8
Medium	36.7	36.5
Heavy	27.8	18.5

Table 6 shows the overall percentage of each infection level for the duration of the survey. Microgemma sp. was found to occur most frequently at medium infection levels with equal proportions of heavy and low infection levels. Hemiurid metacercaria on the other hand unable to multiply in their host occur predominantly at low and medium infection levels. The percentage of heavily infected fish being similar to that of fish with no infection. It must remembered however, that these categories of infection levels are arbitary and levels of one parasite species should not be compared too closely to those of another.

In addition to the investigations of incidence, mean intensity levels were also examined for both Microgemma sp. and hemiurid metacercaria. Seasonal variation in mean infection levels (figs.80,81) are shown by the combined results of 30 months data. Continuous plots of mean infection levels for the duration of the survey (figs.82.83) enabled a comparison of consecutive years data. Mean inshore sea temperatures are also shown on these figures to show the relationship if any, with infection level. Figure 80 shows the peak mean infection level of Microgemma sp. 2.5 to coincide with maximum sea temperatures. A decline in

infection occurs in October before rising to 2.25 in November and then dropping to an overwinter level of less than 2. Mean infection drops sharply from 1.75 in March to its lowest level of 0.5 in June.

Investigations of the hemiurid metacercaria in figure 81 show the highest mean infection level to occur in February at 2.1 which declined to 0.76 in early summer. Mean infection levels increased to 1.35 in August and 1.8 in November with a slight drop recorded in September and October.

Infection levels of Microgemma sp. appears to have increased over the study period (fig.82). Low levels of infections less than 1 are recorded in June in 1981 and 1982 with peak levels occurring in late summer and winter. The peak September value in 1981 coincides with the highest mean sea temperature recorded. The high standard error recorded in November 1981 may explain the unexpected low mean infection level.

Infection levels of hemiurid metacercaria during the study period have remained constant overall. Again mean infection level is at its lowest of less than 0.5 in June when the young T.bubalis arrive on shore. Highest infection levels were recorded overwinter. The unexpected high level of infection in June 1981 probably results from the single sample.

Variation of monthly infection levels with age and sex

It was considered that interaction between age with microsporidian infection and sex with microsporidian infection could be important factors in the parasites life

cycle. Hence yearly progression of infection for each age class and sex was examined. Infection levels of hemiurid metacercaria were examined in the same way for comparison.

Variation of Microgemma sp. infection with age

Age groups were investigated for each of the two complete years of study, the year beginning April 1st to coincide with termination of spawning (fig.84). Group 'O' fish showed similar trends in levels of infection for both 1981/82 and 1982/83. Samples of group 'O' fish were unobtainable for the months April and May, due to the fact that larval stages remain offshore in the plankton for this period. By the end of June the majority of juvevile fish have returned to the rockpools and the mean infection level with Microgemma sp. is less than 1. This level rapidly rises to 2.5 in August and September before declining in early autumn. A second peak of infection occurs in November/December which drops to a winter level of 1.6 before rising again towards the end of the spawning season in March.

Group 'I' fish show similar mean monthly infections to group 'O' although data for April and May is now available. Mean infection levels drop from 2 in April to 1 in June, the lowest mean value for the year. The following rise in mean infection level is steadier than that of 'O' group fishes, reaching a maximum of 2.35 in autumn 1982. Levels of infection are fairly constant over winter at just below 2 and then rise in spring to a mean level of 2 before declining. (Absent standard error of means in early spring 1981/82 indicate that only one or two samples were available). Data for age group 'II' fish was proportionally

less than that collected for groups 'O' and 'I'. Only the year 1982/83 provided sufficient information to compare monthly variation with previous age groups. A rise in mean infection levels in August to 1.75 from 1 in June and July was followed by a fairly constant infection level over winter before a decline in spring to 1. Generally mean levels of infection from group 'II' fish were lower than for other age groups studied.

Variation of hemiurid metacercarial infection with age

Mean infection levels of hemiurid metacercaria (fig.85) were generally found to be lower than those of Microgemma sp. in the number of infection foci recorded here. Age group 'O' fish show an initial rise in mean infection level after arriving on shore to 1.8 in August 1982. This level declined in autumn before rising to 2.15 in February, its highest level. The high mean infection level for November 1981 was obtained from one fish only and so was not included in the curve.

Age group 'I' fish show a decline in mean infection level from approximately 1.5 in spring to less than 1 in early summer. A subsequent rise in mean infection level to 2 in July was proceeded by a small dip and then a rise to 2.6 in October. Although plots for 1981/82 and 1982/83 are out of phase their overall trend is similar.

The sparse data available for age group 'II' fish allows only a rough comparison with age groups 'O' and 'I'. A low summer level was followed by a rise in the autumn as maintained by groups 'O' and 'I'.

From the above studies, variations between mean infection

levels for different age groups were recognised. By plotting a continuous curve (figs.86, 37) for each generation over the sample period, it is possible to examine the effects environmental factors and initial infection levels on infection trends. Infection levels with Microgemma sp. (fig.86) are initially high and age group 'O' fish generally have the highest infection levels, followed by group 'I' and then group 'II'. However the rises and falls in infection level are similar in each age group. Levels of hemiurid metacercaria (fig.87) appear to follow the generation through, although age group 'II' have noticeably lower infection levels than groups 'O' and 'I'. Initial infection level appears to have a greater influence on metacercarial infection levels than Microgemma sp.

Variation of Microgemma infection with sex

The overall trend of Microgemma sp. infection over the year (fig.88) is similar for both sexes of T.bubalis. However it is noticeable that the peaks and troughs described by the male population are more exaggerated than those of the female which generally exhibits lower levels of infection. The mean January infection level is 0.5 for the female host and 1.0 for the male. This rises to 2.105 in the spring for the female and 2.55 in the male. In summer the mean infection level for the female drops to 0.55 whereas in the male it drops to 0. The summer peak is again higher for the male than the female by 0.5. The female population complete the year with a lower mean infection level than that of the male.

Variation of hemiurid metacercaria with sex

Hemiurid metacercaria like Microgemma sp. show similar seasonal variation for both sexes of the host (fig.89). The greatest difference between the sexes occurring in January, when a mean infection level of 2 is recorded for the male population and 0 for the female population. The mean female level then rises to just below 2 in February and levels off at 1.4 in spring. The mean male population infection level drops in stages from a February peak of 2.0 to a summer level of 1. A rise in mean infection level in late summer reaches 2 for both the male and female population. By the end of the year the female population has a lower mean infection level than the male.

Age, sex combinations

The effect of age and sex on infection levels have been examined independantly for both hepatic parasites. Now the effect of age and sex together are investigated. Ninety five per cent confidence limits were used to illustrate the differences between the six age/sex combinations (fig.90). These were group 'O' male, group 'O' female, group 'I' male group 'I' female, group 'II' male, and group II female. Presence of infection was taken to include medium and heavy levels of infection combined. Chi-square analysis (Appendix 2) carried out to confirm exclusive confidence limits of Microgemma sp. infection with the various age/sex combinations gave a value of 18.72 ($P<0.05$). This was entirely due to the exclusive values of group 'O' male and group 'II' female. Although infection levels of group 'I' male appeared exclusive from group 'O' male on analysis it was not found to be so. The hemiurid metacercaria did not show significant variation in infection levels between the various age/sex combinations.

Statistical analysis of parasite/host interaction at Portwrinkle

The aim of this study was to investigate possible interaction between host and parasite comparing weight, length, condition factor, age, sex, liver weight, gonad and lipid content of the liver with the intensity of infection with the microsporidian Microgemma sp. and hemiurid metacercaria (Appendix 2). Investigations were carried out on the same sample of 241 fish collected from Portwrinkle. Although parasites other than those mentioned above were found in the tissues outside the liver. They occurred in low numbers and were assumed here to have an insignificant effect on the condition of the host. Species recorded included Pleistophora sp. and juvenile nematodes. Investigations were not made of the intestinal and luminal species, in view of their low degree of pathogenicity assumed by this group in fish, (Smythe J.D 1962).

The interactions of Microgemma sp. with the host T. bubalis

Chi-square analysis of length, weight and age of host with microsporidian infection (fig.91) gave significant results of 38.23 ($P<0.05$), 40.61 ($P<0.05$) and 16.9 ($P<0.05$) respectively. Contributions to each of these values followed in similar patterns indicating an expected relationship between length, weight and age. On one hand small, group 'O' fish, contrary to the expected pattern fell chiefly into two categories, uninfected and heavily infected whereas on the other hand large, group 'II' fish although having more infected members also had far less heavily infected members than expected. Investigations into interactions between sex and microsporidian infection showed male

fish in contrast to the females to have less than expected uninfected members and more than expected heavily infected members. Chi-square analysis between length and sex indicated that there were more than expected female fish greater than 7.6cm and fewer than expected males ($P<0.05$). However the chi-square value of sex with age was not significant ($P<0.05$), indicating that sex ratio did not vary with age. Actual values of sex ratio clearly disagreed with this and on closer examination the chi-square value was found to be only just insignificant with group 'II' fish contributing approximately 80% to the value. It is thought that the age span of the divisions were too large to show up sexually selective mortality thought to occur in age group 'I' and so smaller divisions on age such as months would be required to expose changes in sex ratio.

Condition factor, taken as a measure of fitness did not provide a significant result with microsporidian infection, neither did liver percentage body weight or lipid percentage liver weight, indicating that infection did not seriously affect host condition. Gonad percentage body weight did not give a significant chi-square value with microsporidian infection.

The interactions of hemiurid metacercaria with the host T.bubalis.

Similar results were obtained with metacercarial infection as with microsporidian infection (fig.92). Length, weight and age all gave significant chi-square values of 44.1 ($P < 0.05$), 49.57 ($P<0.05$) and 20.8 ($P<0.5$) respectively. Condition factor, sex, liver percentage body weight and lipid percentage liver weight did not produce significant

chi-square values, indicating no interaction with the level of parasitization of hemiurid metacercaria. However gonad percentage body weight did give a significant chi-square value of 21.04 ($P<0.5$) with infection. A higher than expected number of heavily infected fish had small gonads and fewer than expected heavily infected had large gonads, otherwise contributions were contradictory and no overall trend was seen (fig.84).

Interactions between Microgemma sp. and hemiurid metacercaria

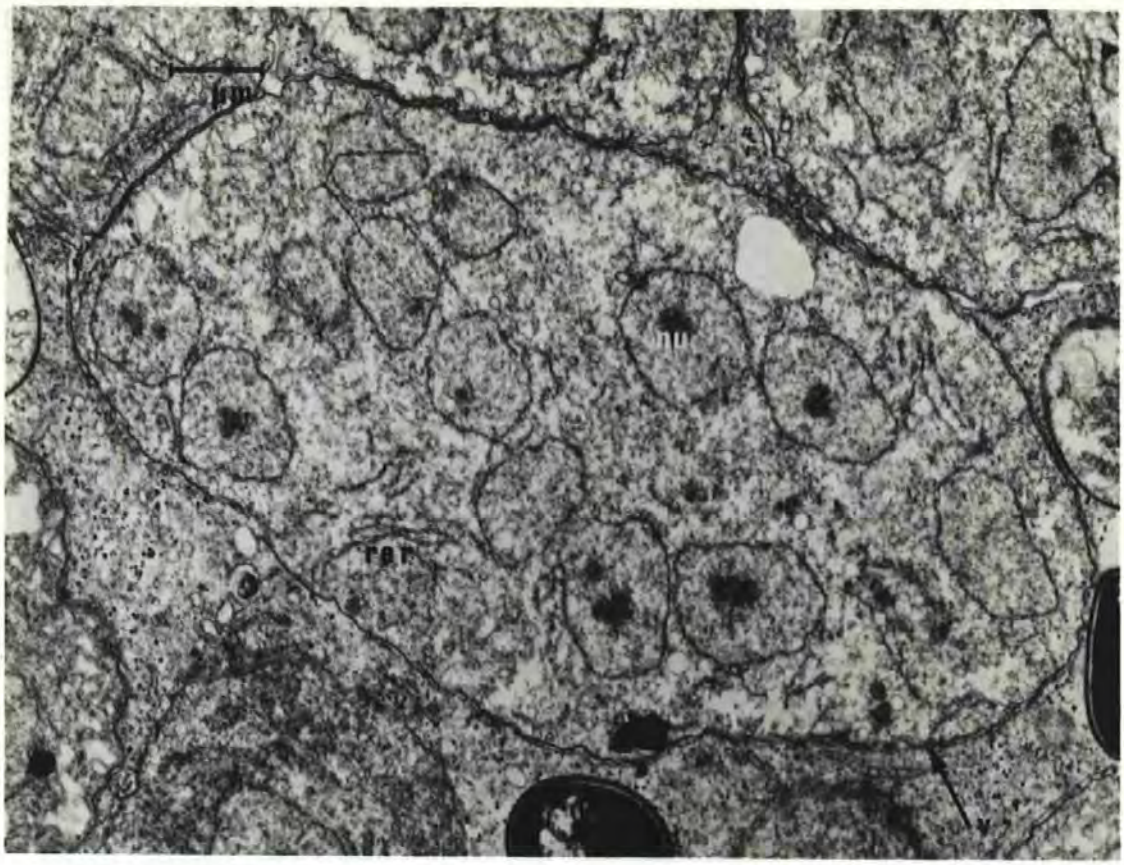
Chi-square analysis of interactions between Microgemma sp. and hemiurid metacercariae (fig.90) proved significant ($P<0.5$) at 21.59. More fish were infected with hepatic parasites than expected and fewer fish had heavy microsporidian infection and no metacercarial infection than expected. Almost every case of heavy microsporidian infection was accompanied by a higher than expected level of metacercaria. Absent and present microsporidian infection were generally accompanied by less than expected metacercarial infection. (It is important to remember here that whereas microsporidia may multiply in the host, metacercaria do not). Light microscope sections show the distribution of encysted metacercaria and xenomas (figs.55, 56).

PLATE ABBREVIATIONS

ad	anchor disc
cy	cytoplasm
ccy	cell cytoplasm
ch	chromatic material
cb	cell boundary
c	collagin
er	endoplasmic reticulum
g	glycogen
eb	electron dense body
h	host
i	inclusion body
iccy	infected cell cytoplasm
jc	junctional couple (complex)
ld	lipid droplet
lp	lamellar polaroplast
m	mitochondria
mb	manubrium
mbr	membrane
me	meront
mp	macrophage
mv	microvilli
n	nucleus
nu	nucleolus
nx	necrotic xenoma
pl	polaroplast
pv	posterior vacuole
p.ve	polar vesicle
p.f	polar filament
sp.	spore
spb	sporoblast
spt	sporont
uccy	uninfected cell cytoplasm
r	ribosomes
spl	spindle plaque
vcy	vesicular cytoplasm
x	xenoma

Figure 9 Multinucleate meront of Microgemma sp. from the liver of Taurulus bubalis showing 13 nuclei. Note the membrane bound vacuole.

Figure 10 Multinucleate meront of Microgemma sp. from the liver of Taurulus bubalis to show cytoplasmic constrictions (arrowed) possibly indicative of plasmotomy. Note the proximity of host mitochondria to vacuolar membrane and dense host cytoplasm.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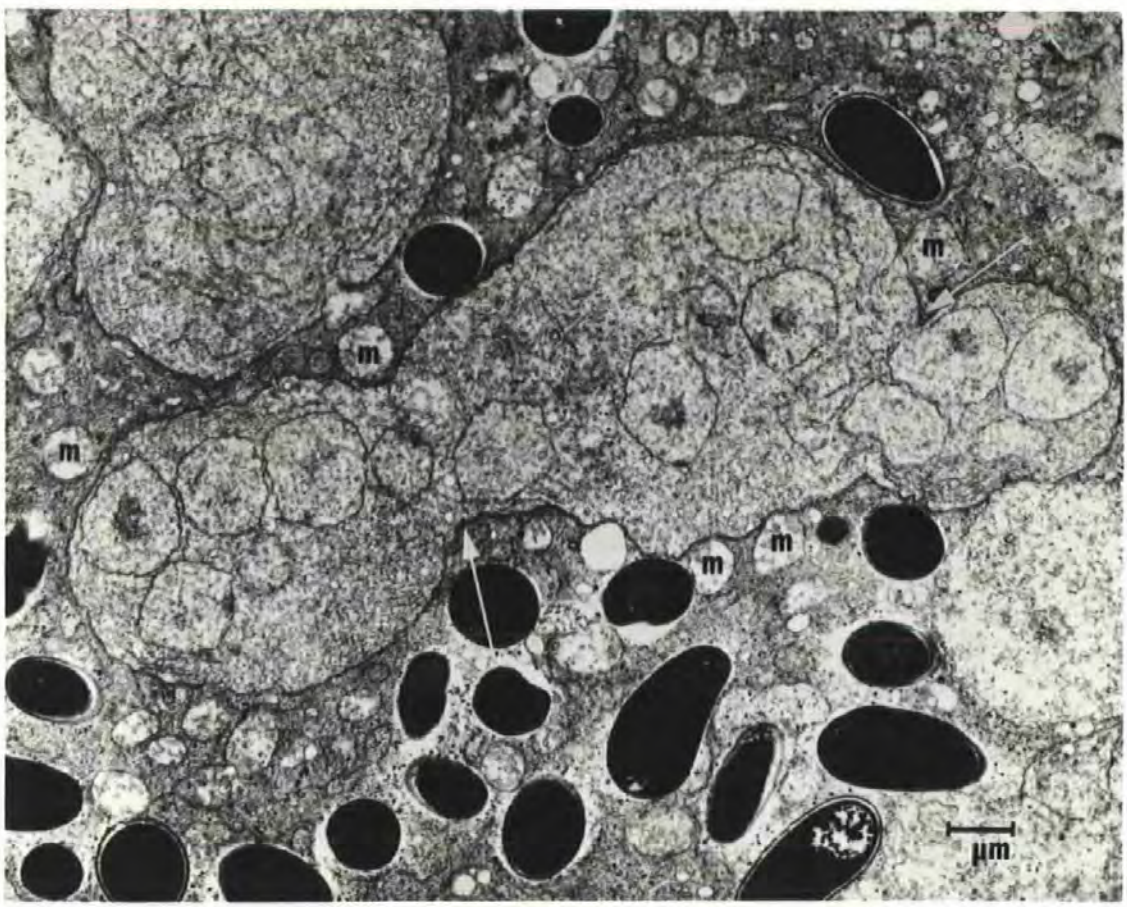
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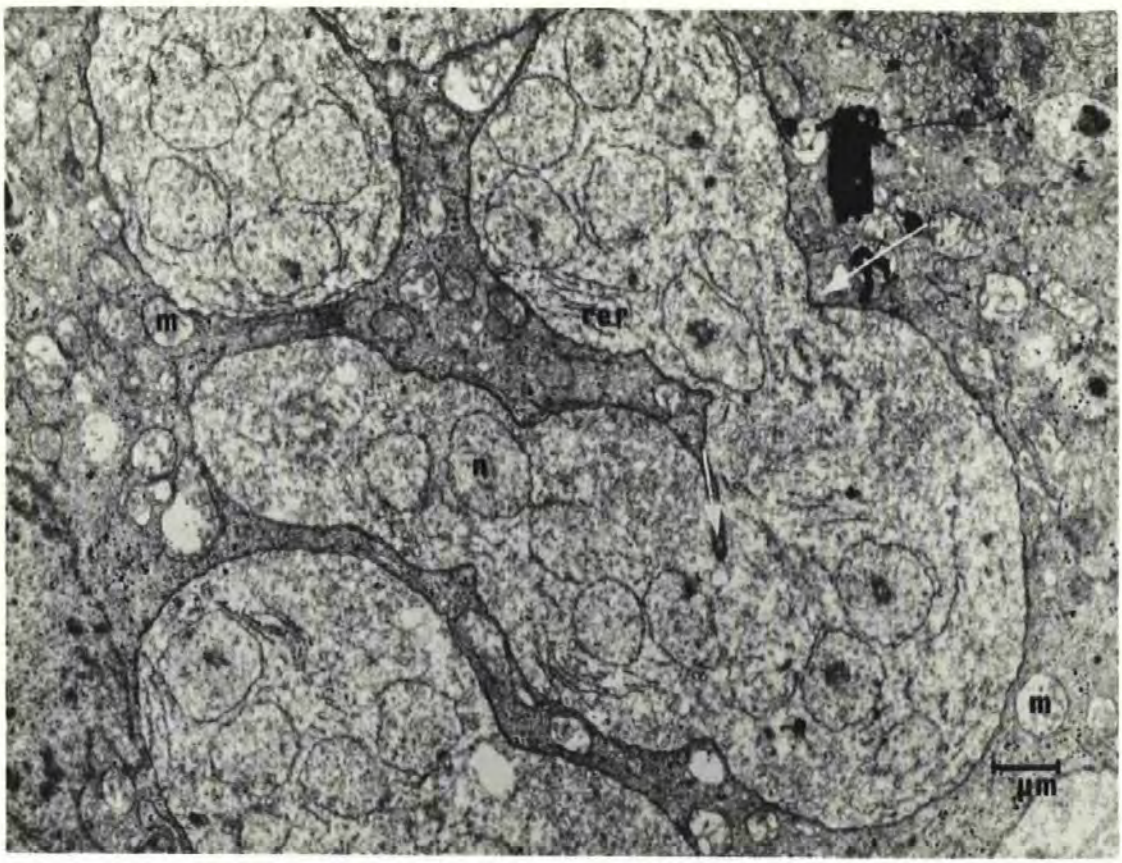
Figure 11 Multinucleate meront of *Microgemma* sp. from the liver of *Taurulus bubalis* showing its elongated irregular shape with deep infolds of cytoplasm (arrowed). Note the adjacent meronts and close proximity to host nucleus.

Figure 12 Spherical meronts of *Microgemma* sp. from the liver of *Taurulus bubalis*, probably following division. Note the prominent vacuolar membrane (arrowed) and rough endoplasmic reticulum.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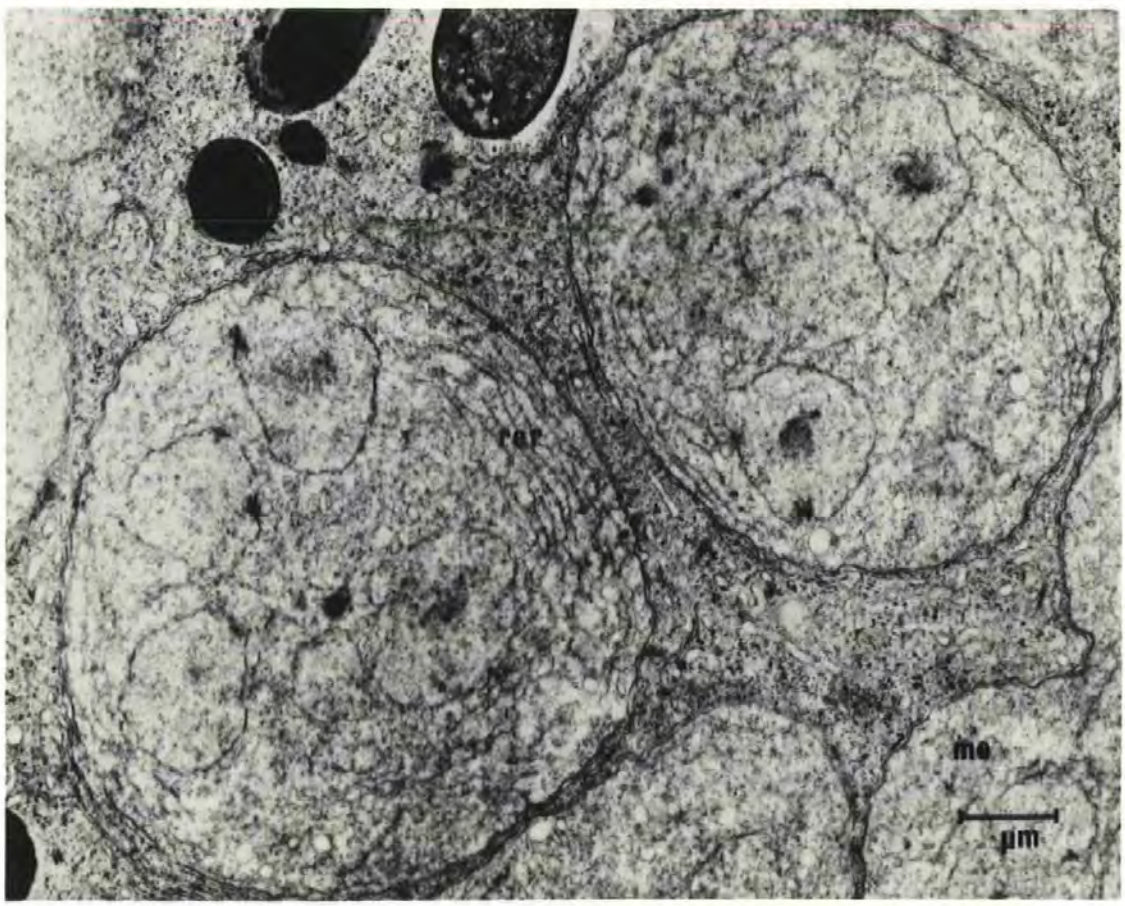
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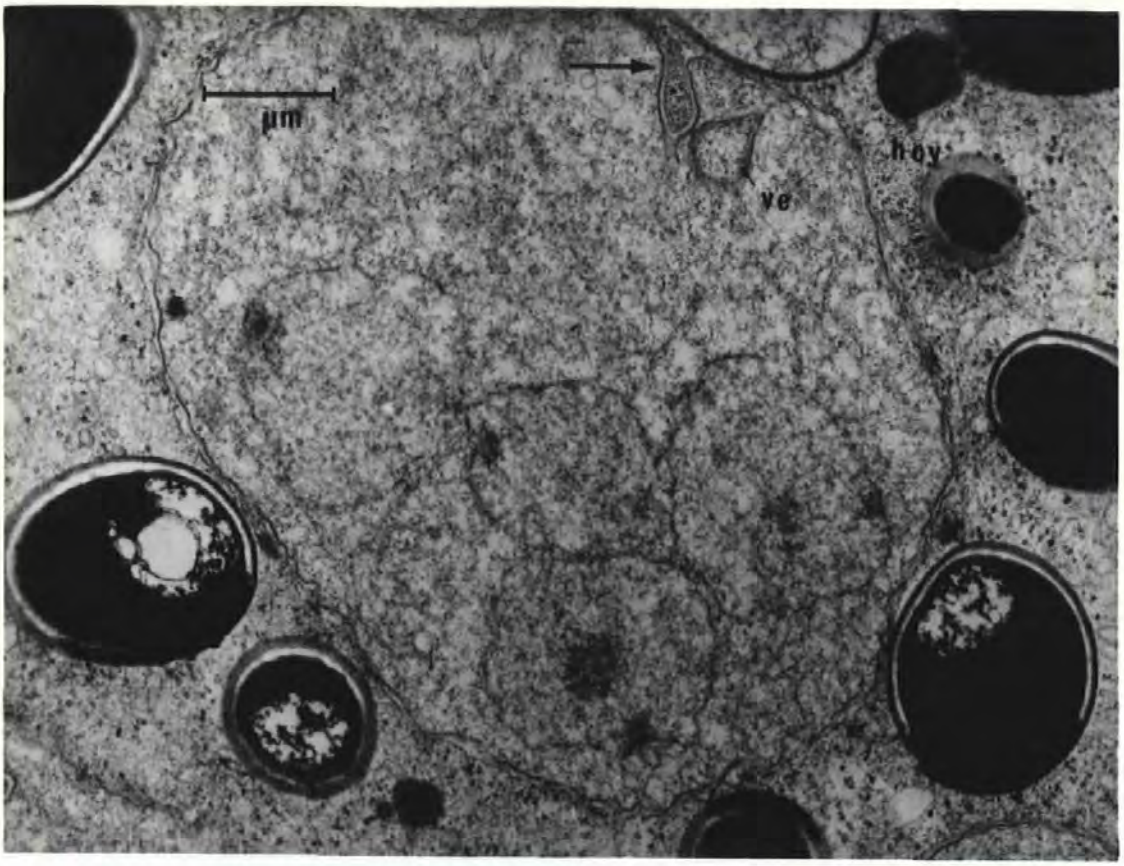
Figure 13 Meront of Microgemma sp. from the liver of Taurulus bubalis. Note the formation of pinocytic vesicles (arrowed).

Figure 14 Meront of Microgemma sp. from the liver of Taurulus bubalis. Host cell, parasite interface to show details of membranes.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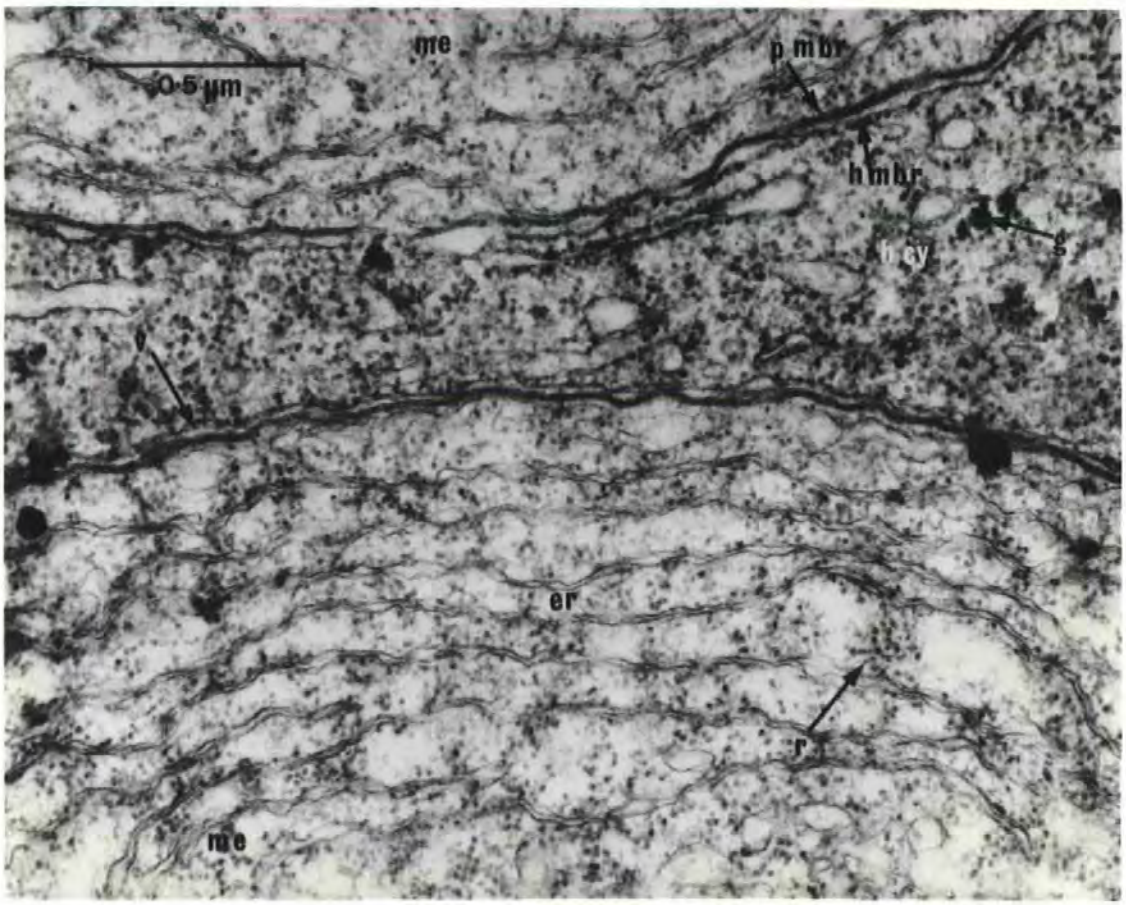
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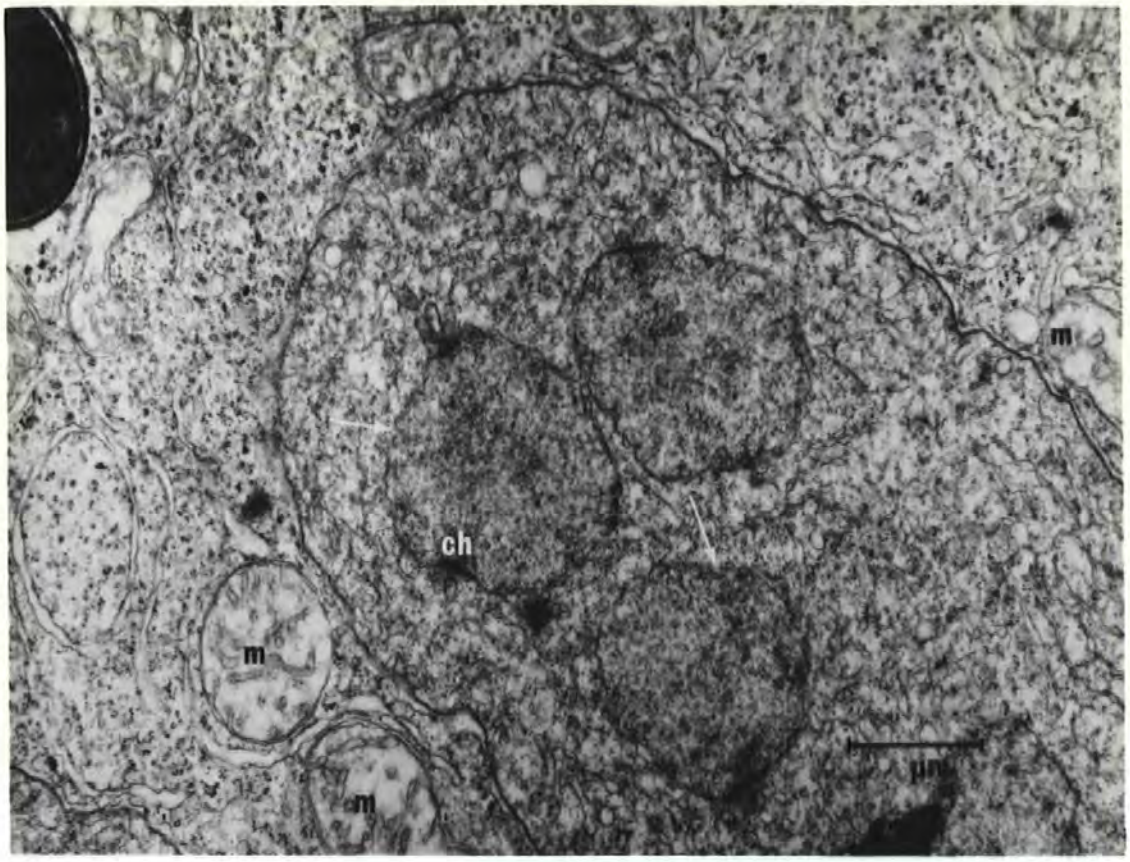
Figure 15 Meront of Microgemma sp. from the liver of Taurulus bubalis to show nuclear pores (arrowed) and chromatic material.

Figure 16 Meront of Microgemma sp. from the liver of Taurulus bubalis to show early stage of nuclear division with chromatic material concentrated at spindle plaque and associated polar vesicles.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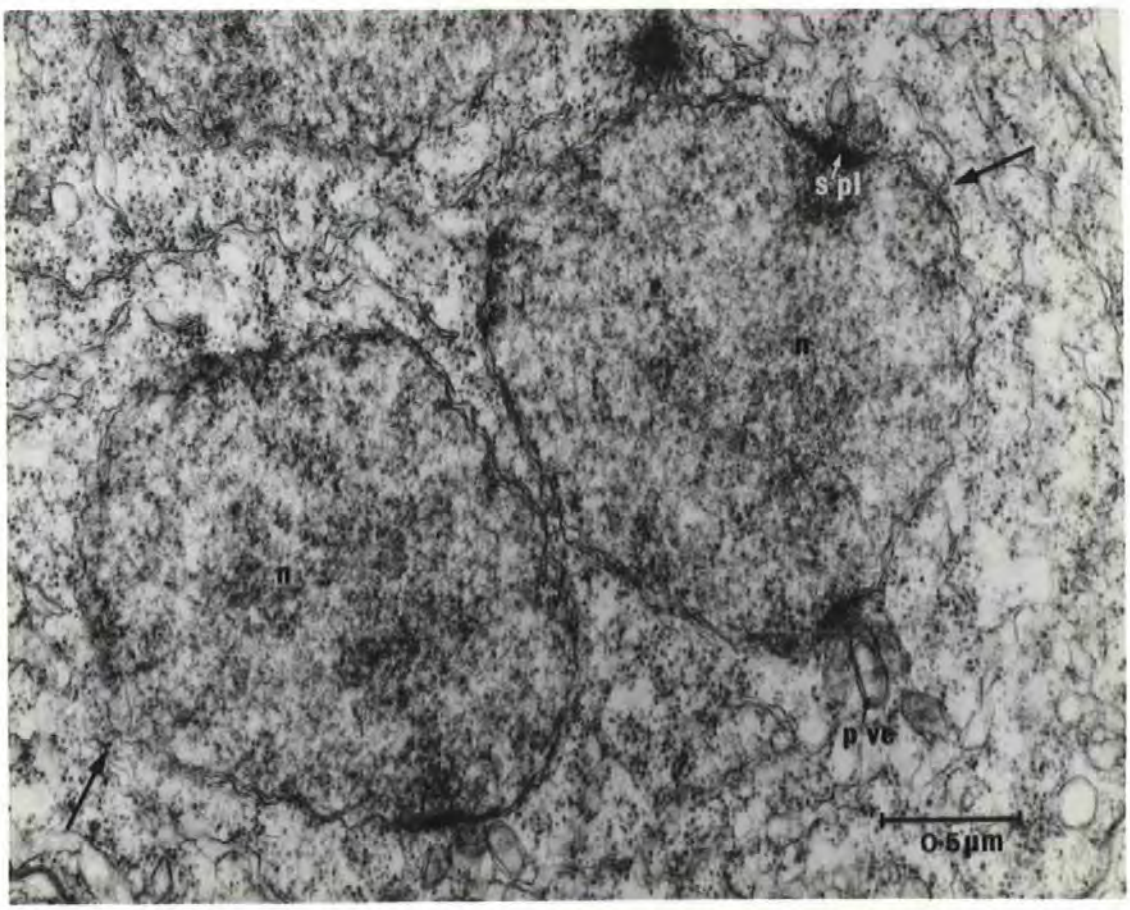
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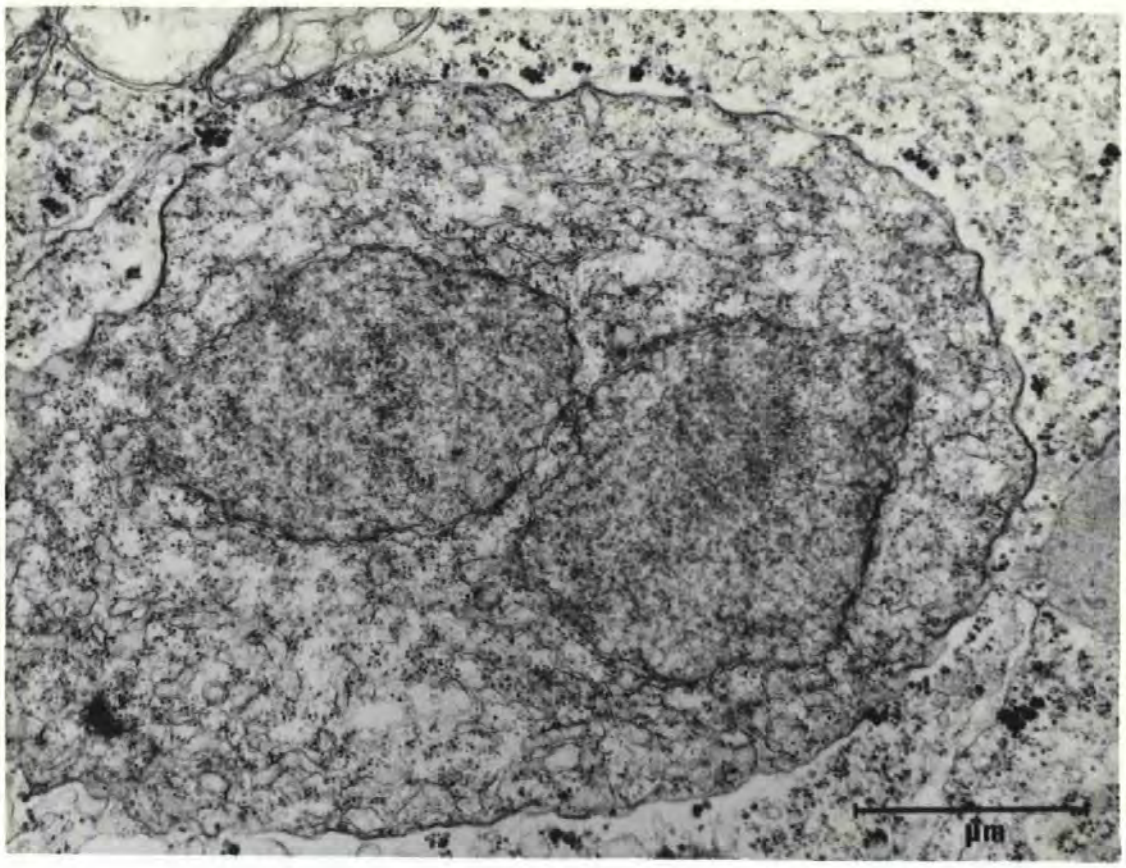
Figure 17 Microgemma sp. from the liver of Taurulus bubalis showing possible intermediate stage between meront and sporont with loss of surrounding host membrane.

Figure 18 Sporont of Microgemma sp. from the liver of Taurulus bubalis. Note the absence of surrounding host cell membrane and deposition of extramembraneous material and the formation of a single bud. The cytoplasm is highly vacuolated.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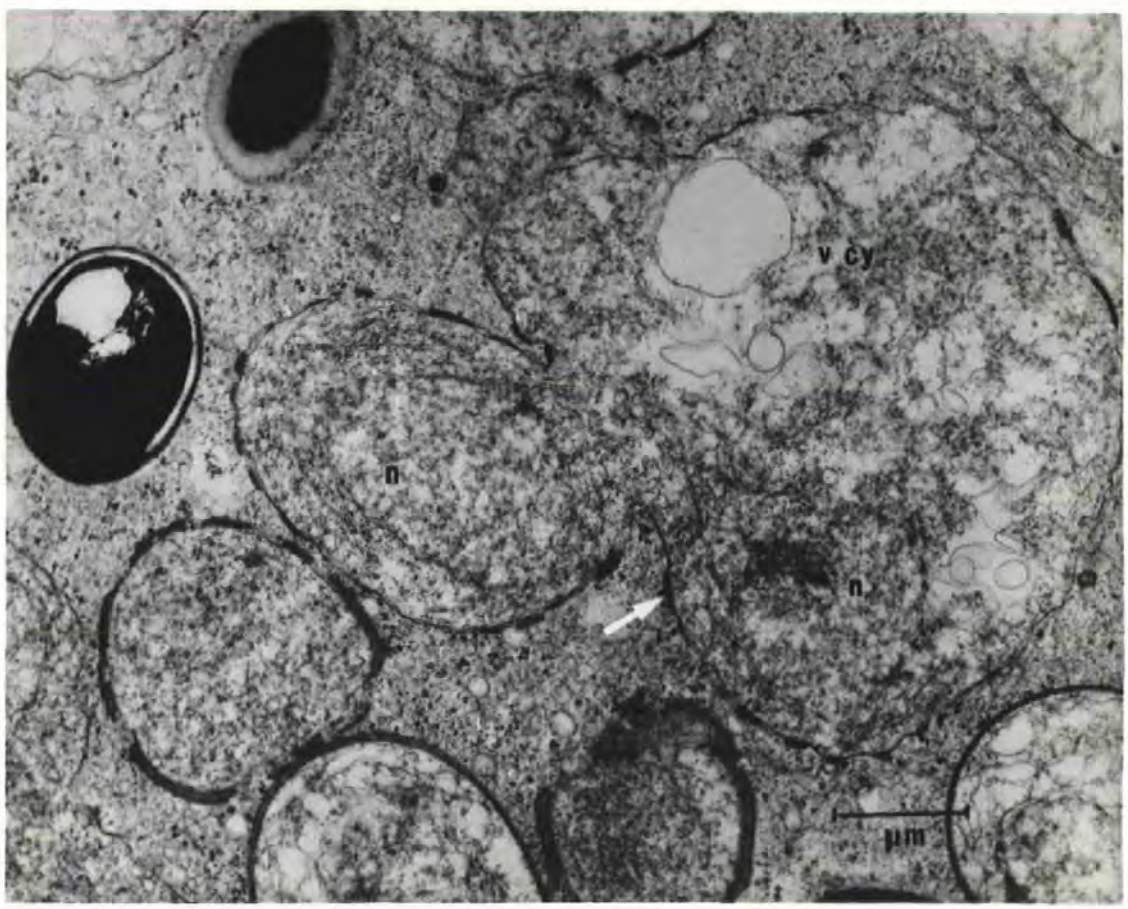
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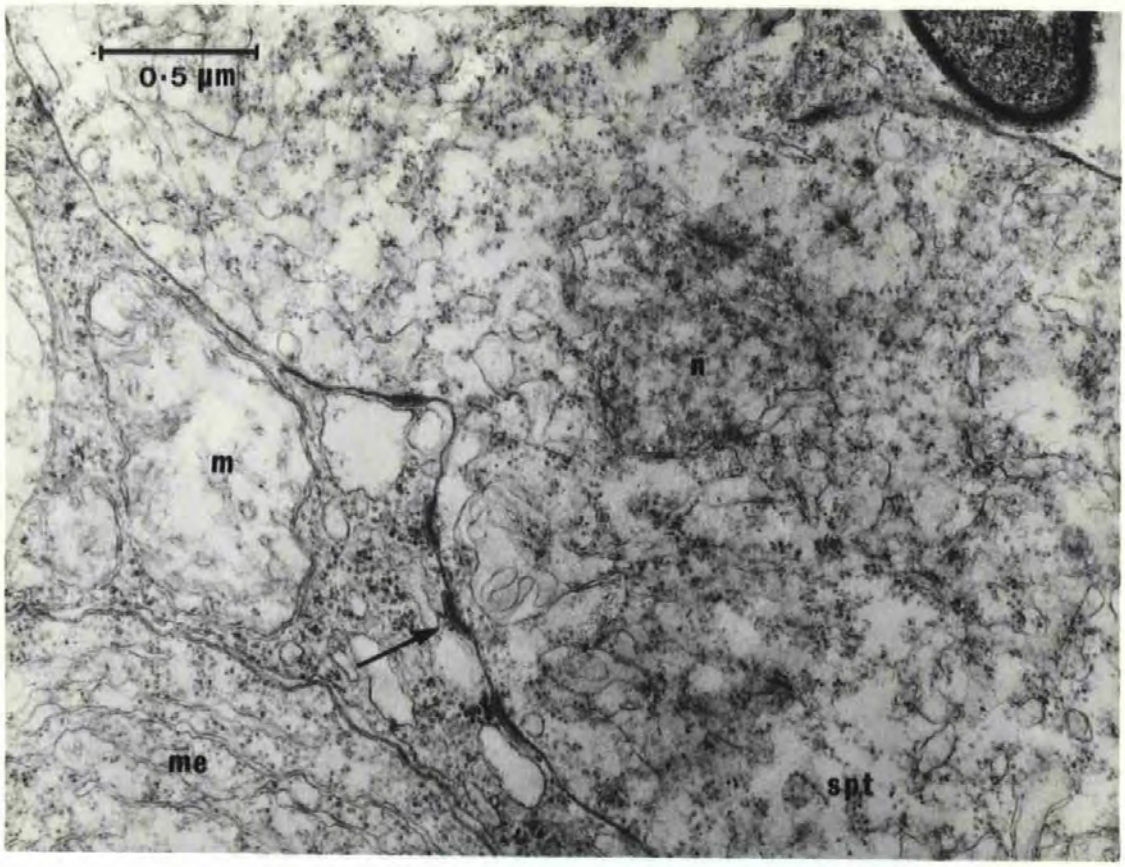
Figure 19 Sporont of Microgemma from the liver of Taurulus bubalis showing the junction of bud formation in greater detail. Note the extramembraneous deposits (arrowed) and adjacent mitochondria.

Figure 20 Sporont of Microgemma sp. from the liver of Taurulus bubalis showing the host parasite interface.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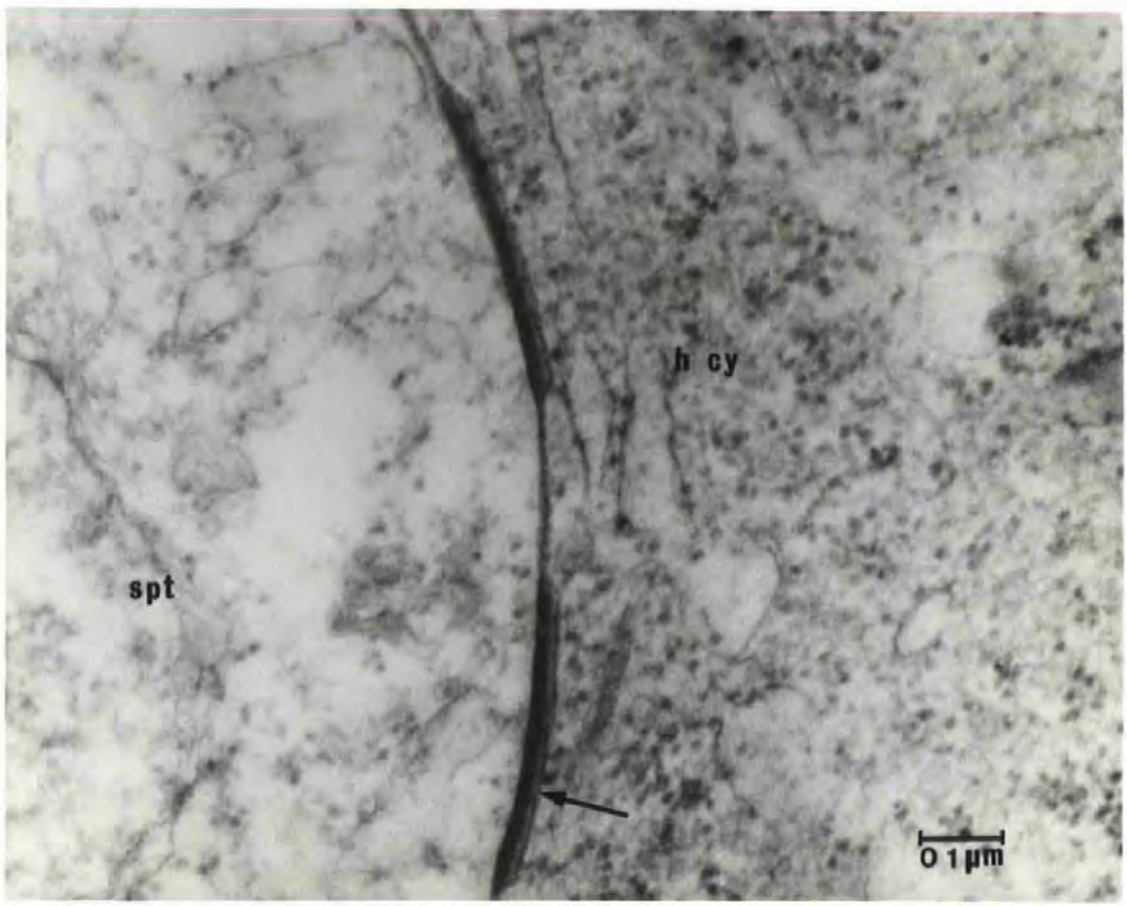
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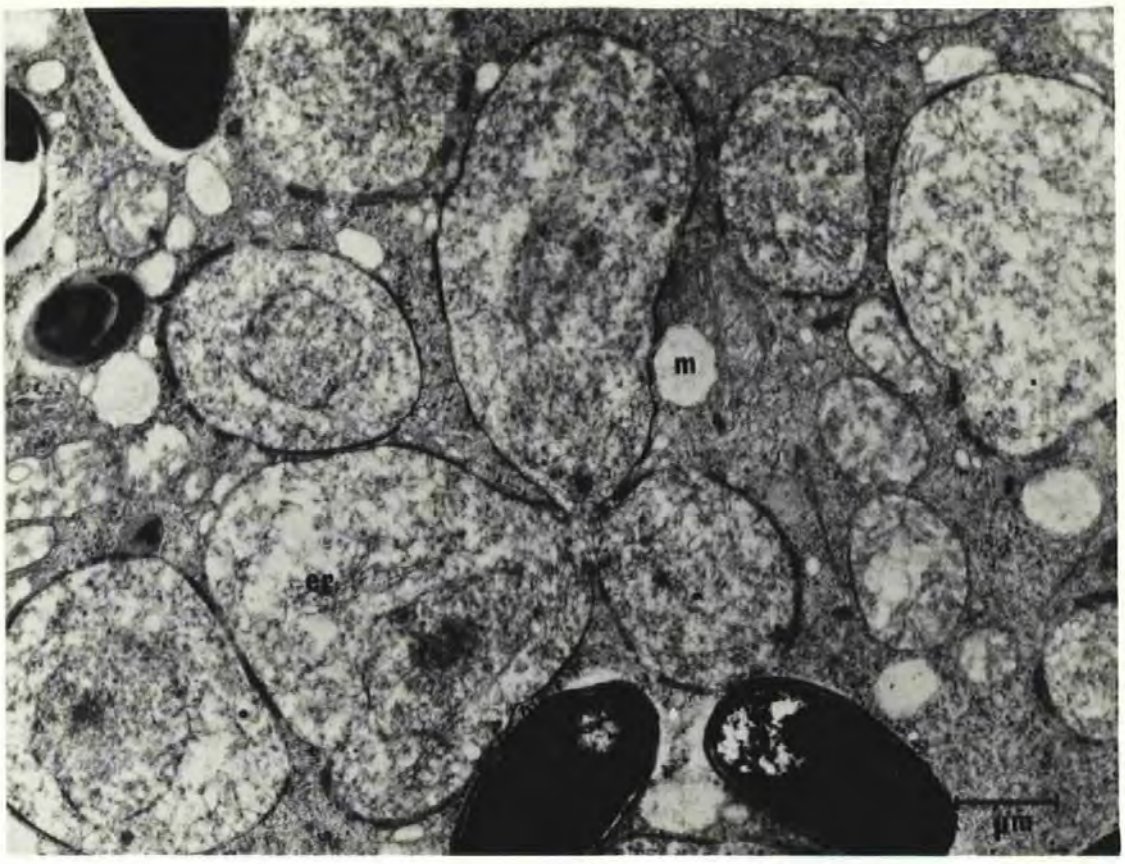
Figure 21 Sporont of Microgemma sp. from the liver of Taurulus bubalis showing advanced stages of budding.

Figure 22 Sporont of Microgemma sp. from the liver of Taurulus bubalis showing rosette form of budding.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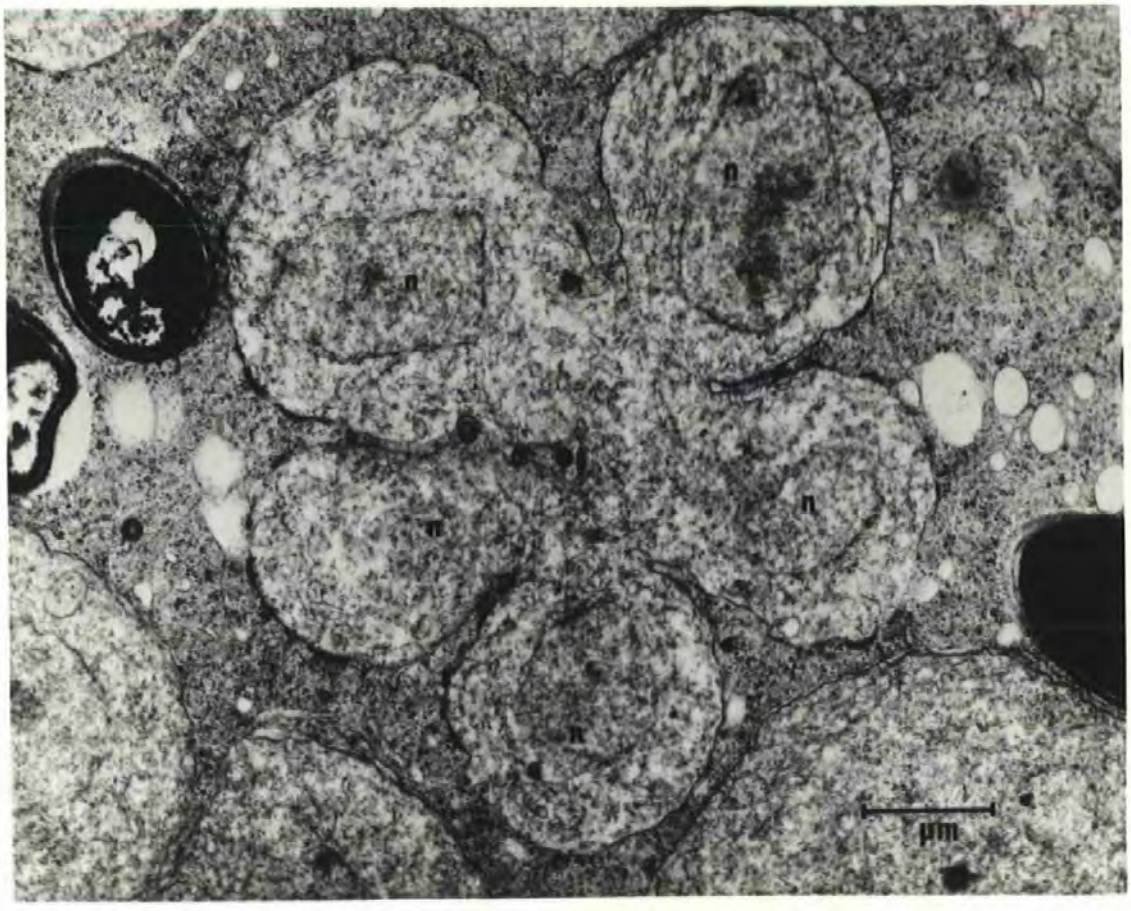
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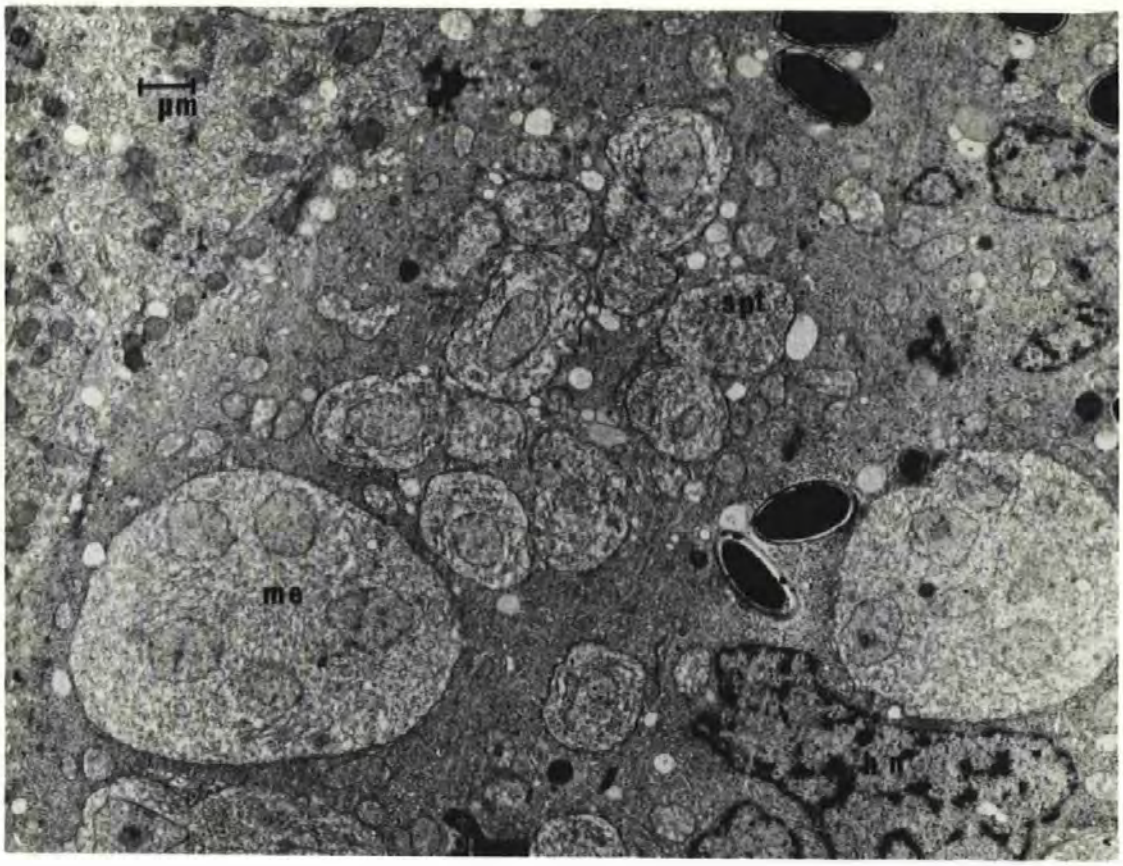
Figure 23 Section of xenoma from liver of Taurulus bubalis showing different stages of development of Microgemma sp. Note the apparent fragmentation of the sporont.

Figure 24 Sporont of Microgemma sp. from the liver of Taurulus bubalis. Note the final stages of division into Mother sporoblast cells and the damaged host mitochondria.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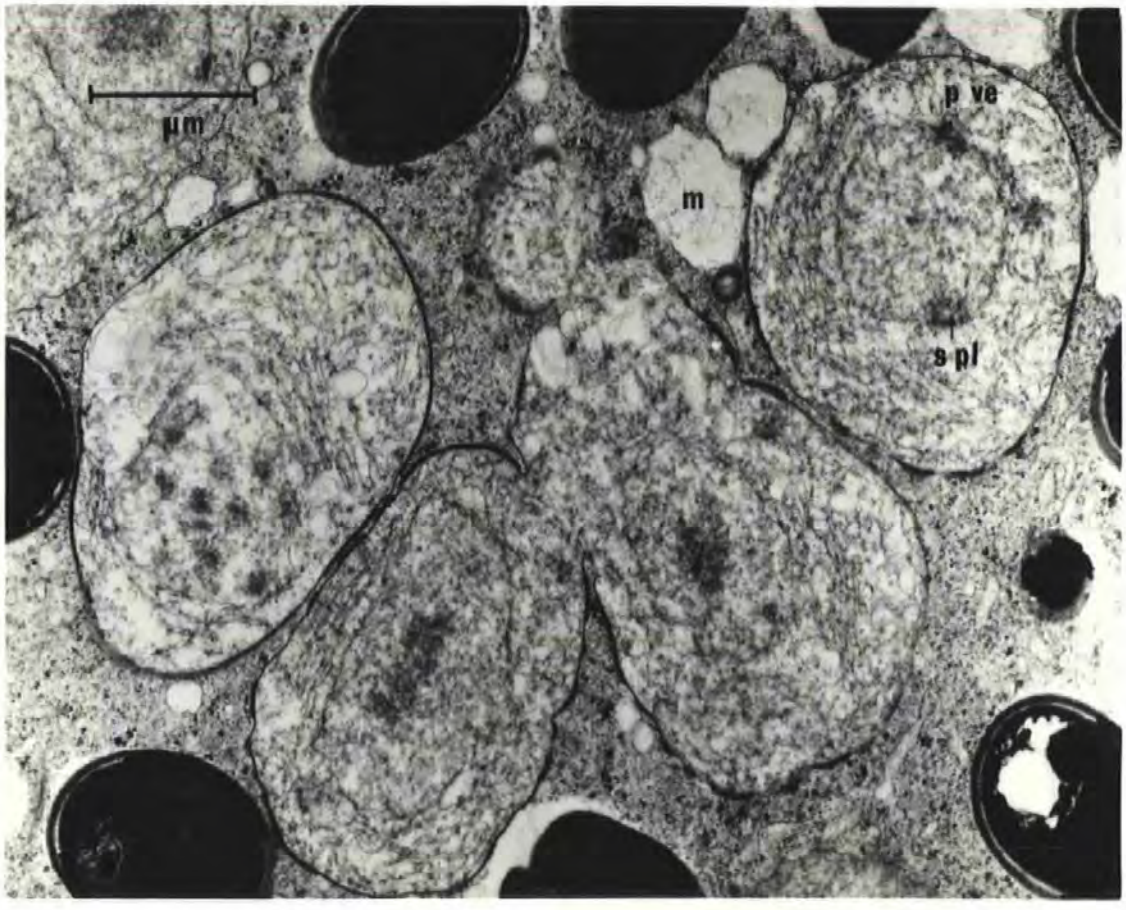
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Figure 25 Mother sporoblast of Microgemma sp. from the liver of Taurulus bubalis showing stages in nuclear division.

Figure 26 Mother sporoblasts of Microgemma sp. from the liver of Taurulus bubalis showing division into sporoblasts.


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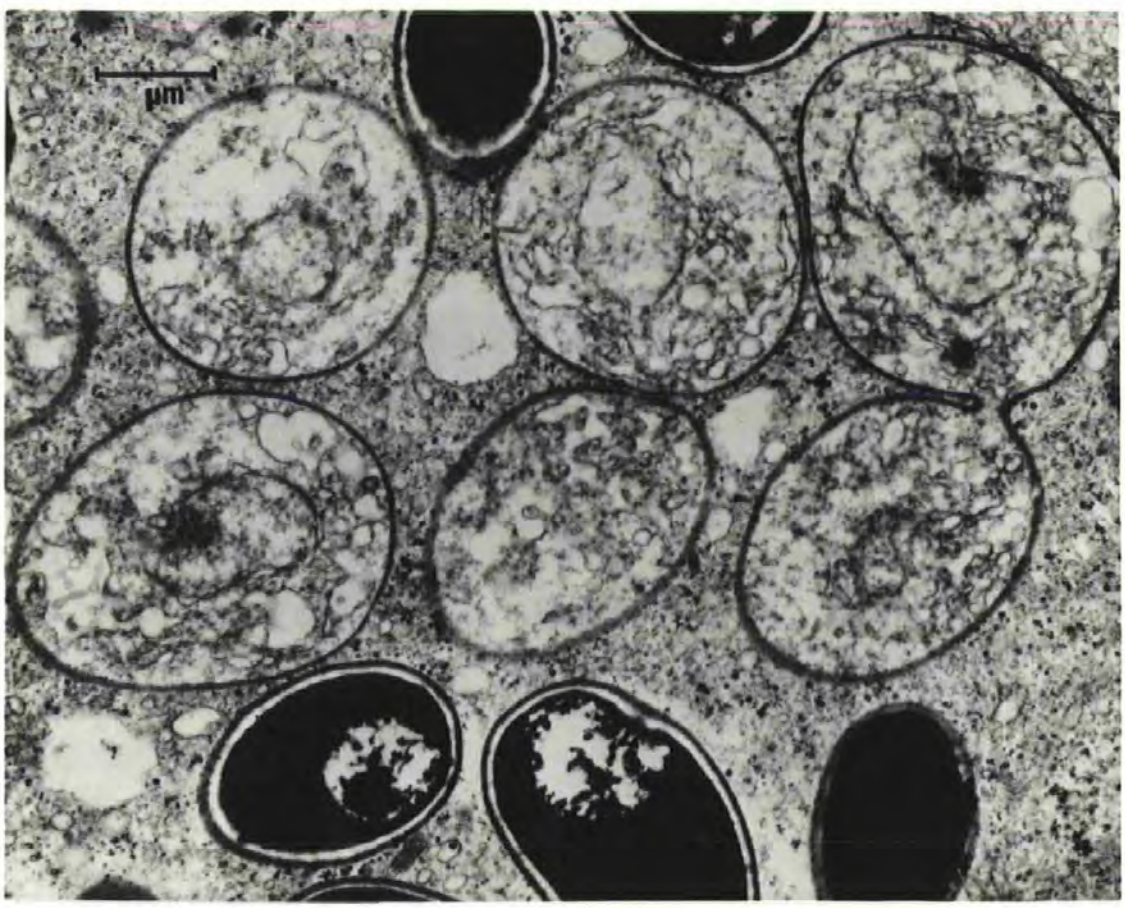
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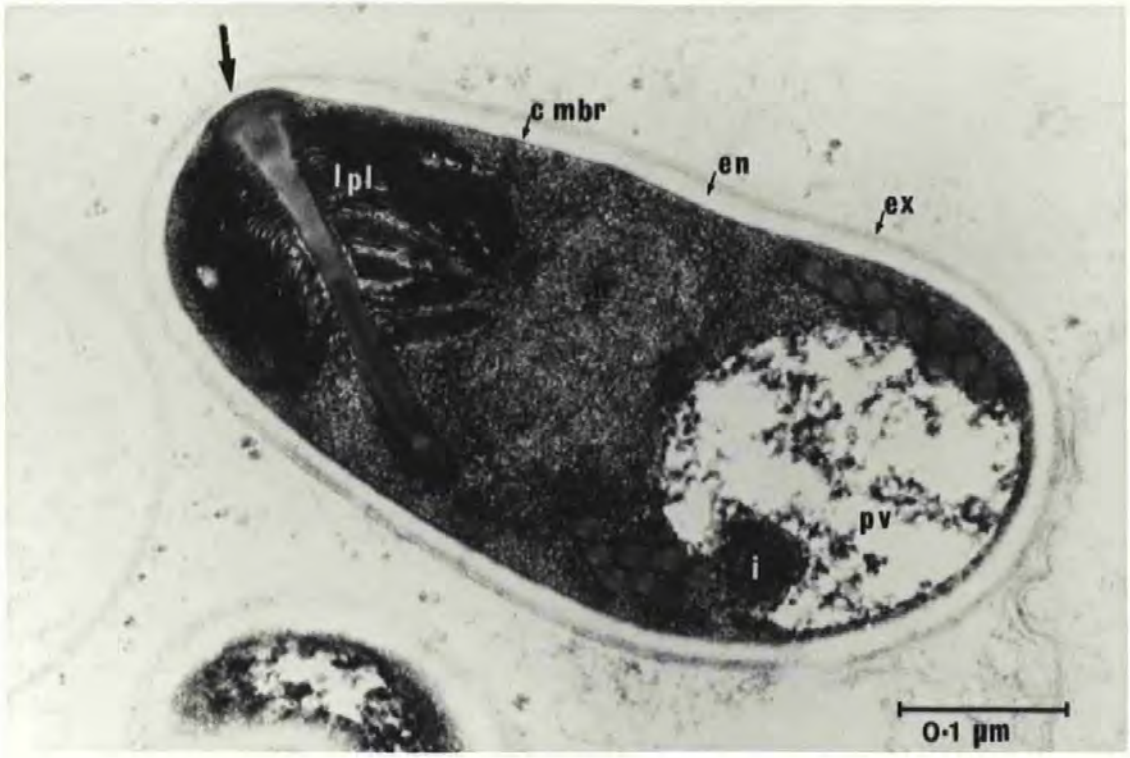
Figure 27 A mature spore of Microgemma sp. from the liver of Taurulus bubalis showing characteristic features, notably the inclusion body within the posterior vacuole, lamella polaroplast and 7 coils of polar filament.

Figure 28 Spore of Microgemma sp. from the liver of Taurulus bubalis showing vesicular and lamella nature of the polaroplast.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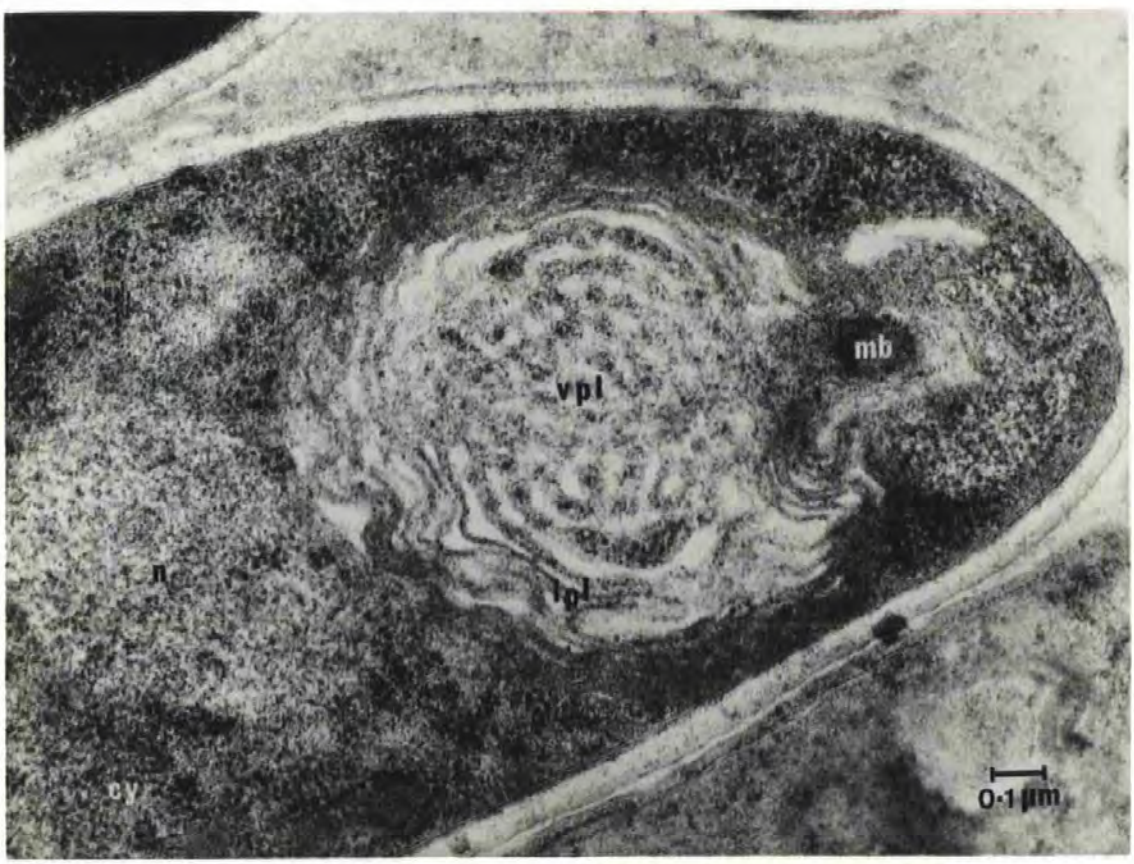
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Figure 29 Scanning electron micrograph of Microgemma sp. from the liver of Taurulus bubalis. Note the asymetrical cross section of the extruded polar filament.

Figure 30 Scanning electron micrograph of Microgemma sp. from the liver of Taurulus bubalis. Note the indentation caused by the collapse of the posterior vacuole.


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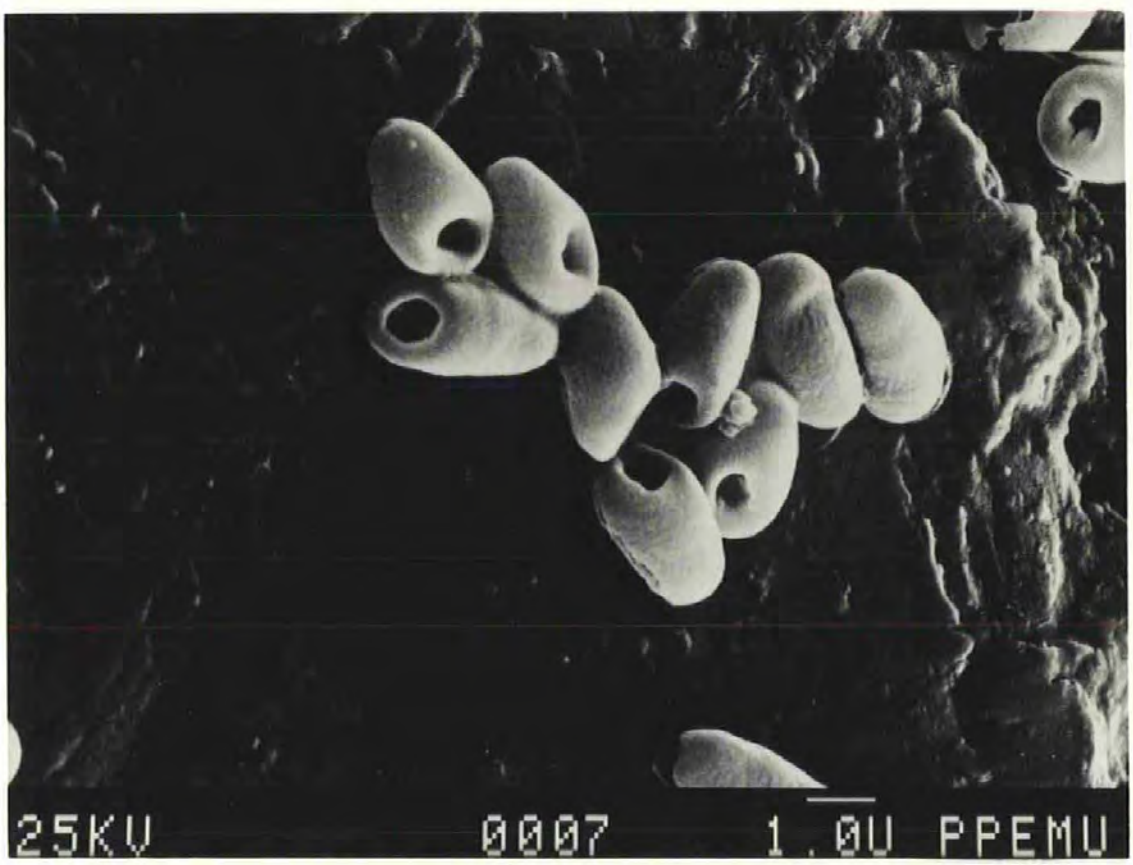
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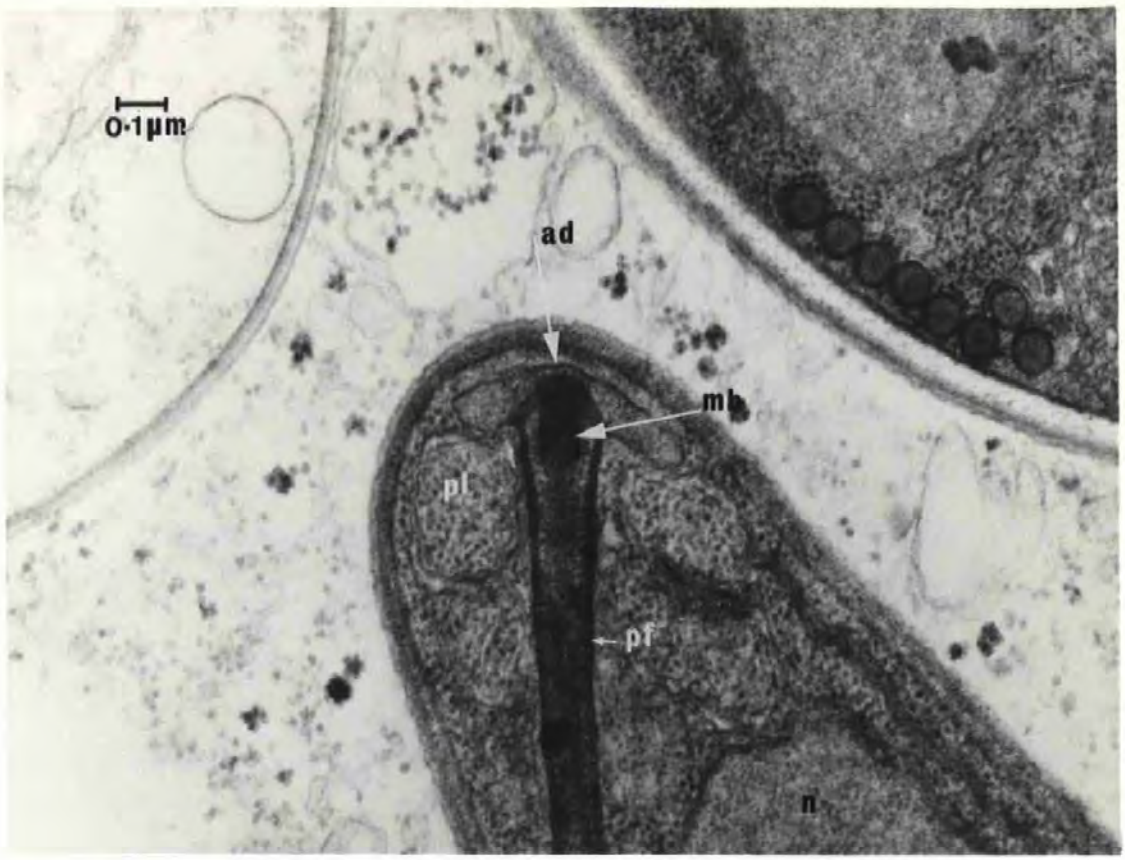
Figure 31 Sporoblast of Microgemma sp. from the liver of Taurulus bubalis. Note the vesicular polaroplast and the well defined anchor disc.

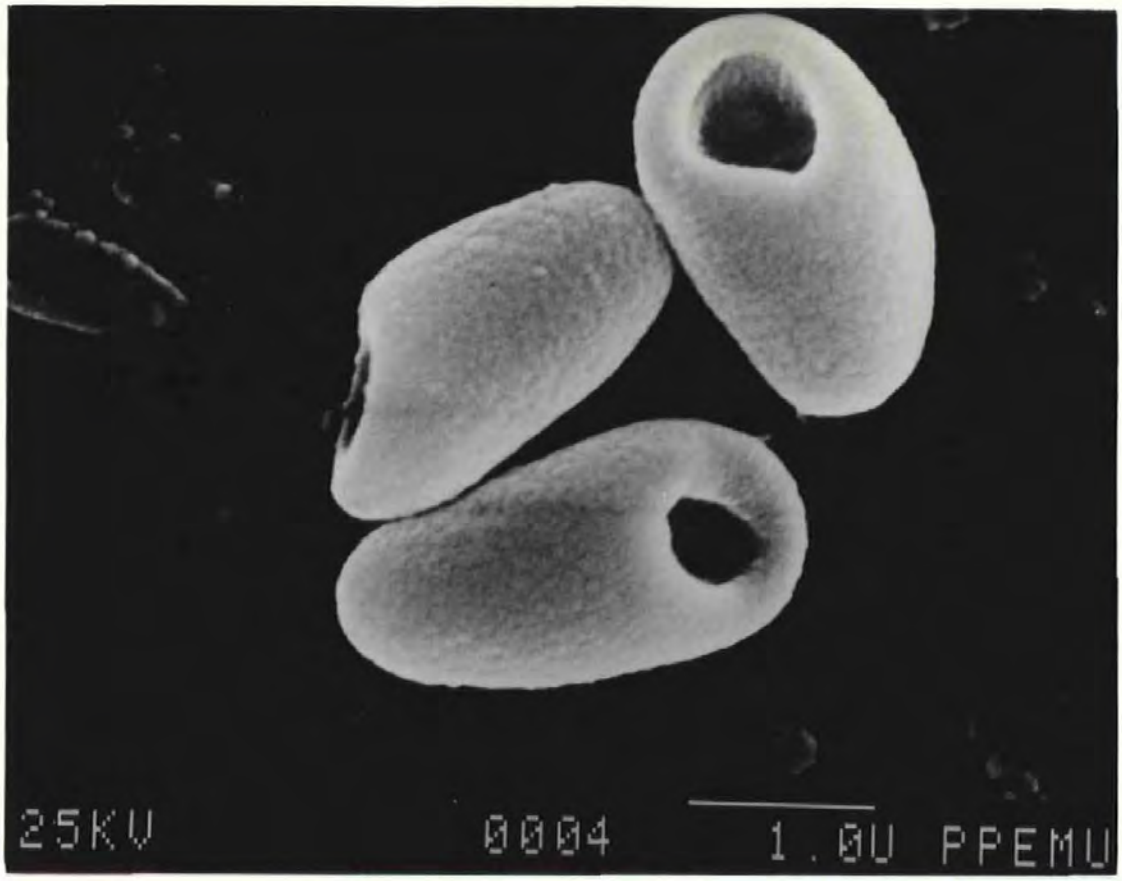
Figure 32 Spore of Microgemma sp. from the liver of Taurulus bubalis. Note the lamella polaroplast.


0·1 µm
ad
mb
pl
pf
n


lpl
0·1 µm

Figure 33 Scanning electron micrograph of spore from Microgemma sp. in the liver of Taurulus bubalis. Note the surface pattern of the spore coat.

Figure 34 Scanning electron micrograph of spore from Microgemma sp. in the liver of Taurulus bubalis. Note the indentation corresponding to the position of the posterior vacuole and a second smaller indentation resulting from fixation.


25KV
0004
1.0U PPEMU

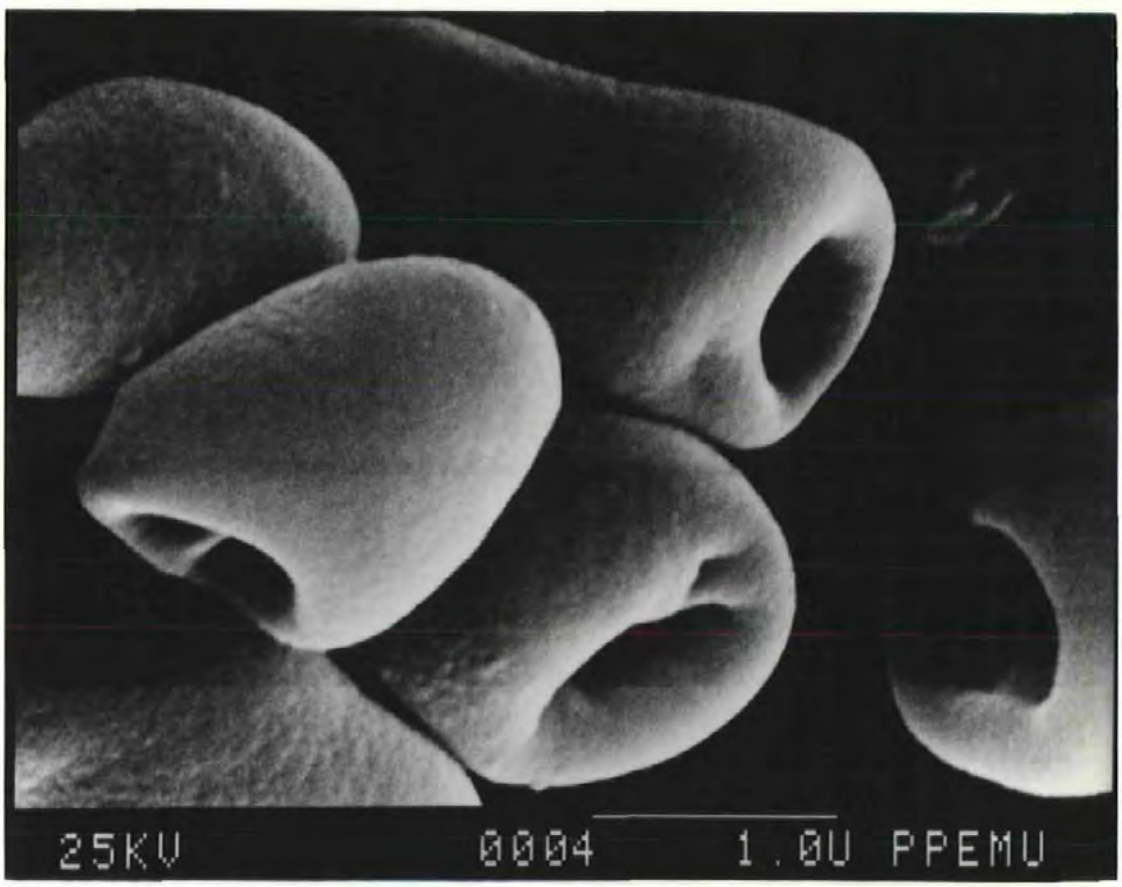
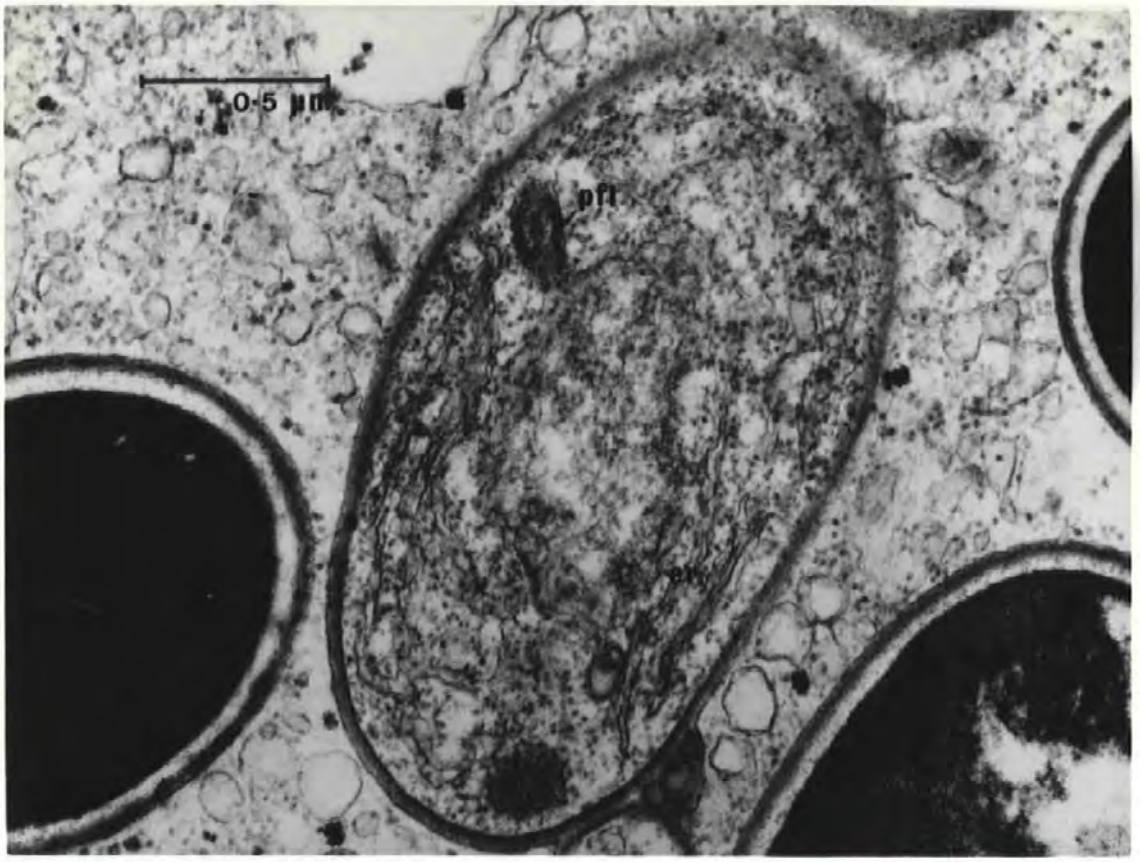

25KV
0004
1.0U PPEMU

Figure 35 Sporoblast of Microgemma sp. from the liver of Taurulus bubalis. Note the vacuolated cytoplasm with prominent membrane and the early stage in development of the polar filament.

Figure 36 Sporoblast of Microgemma sp. from the liver of Taurulus bubalis. Note the formation of the polar filament and the development of the spore coat.


0·5 µm
pfl

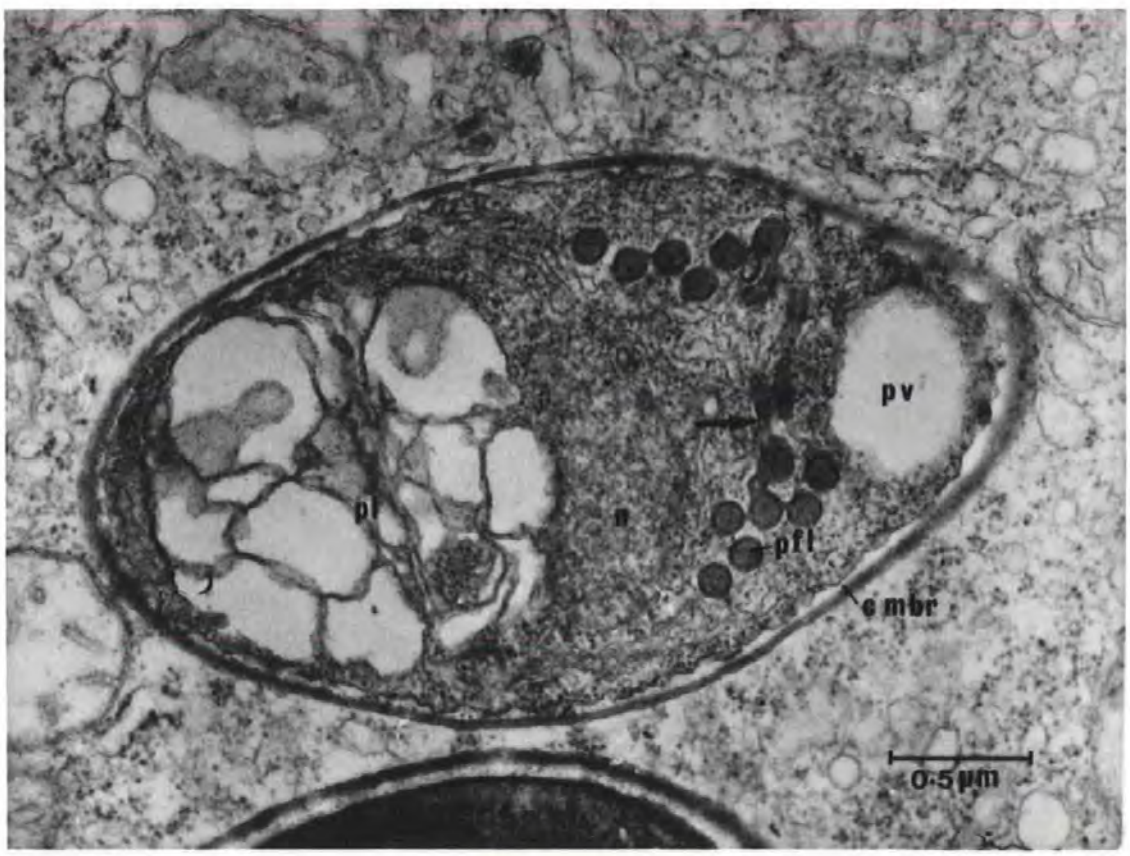
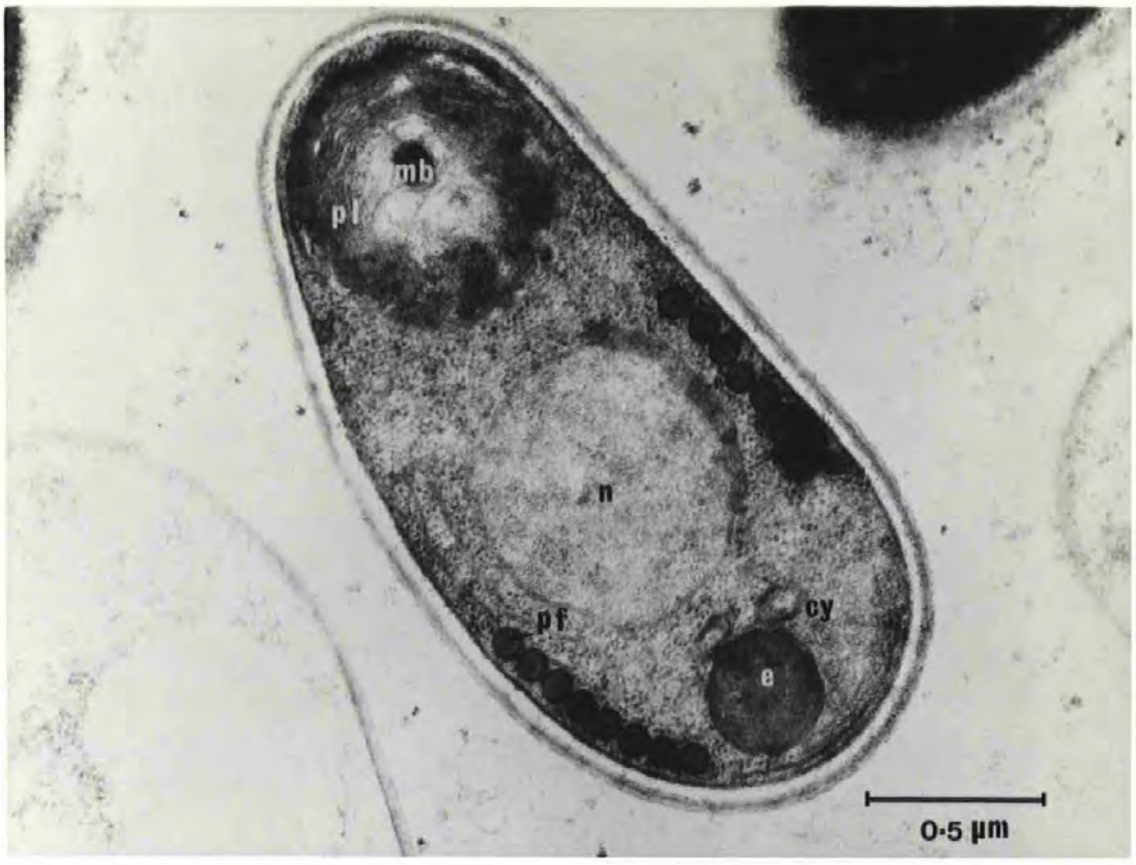

pv
pl
n
pfl
embr
0·5µm

Figure 37 Early spore of Microgemma sp. from the liver of Taurulus bubalis. Note the 8 coils of polar filament, the electron dense body, the absence of the posterior vacuole and the prominent membrane system around the nucleus.

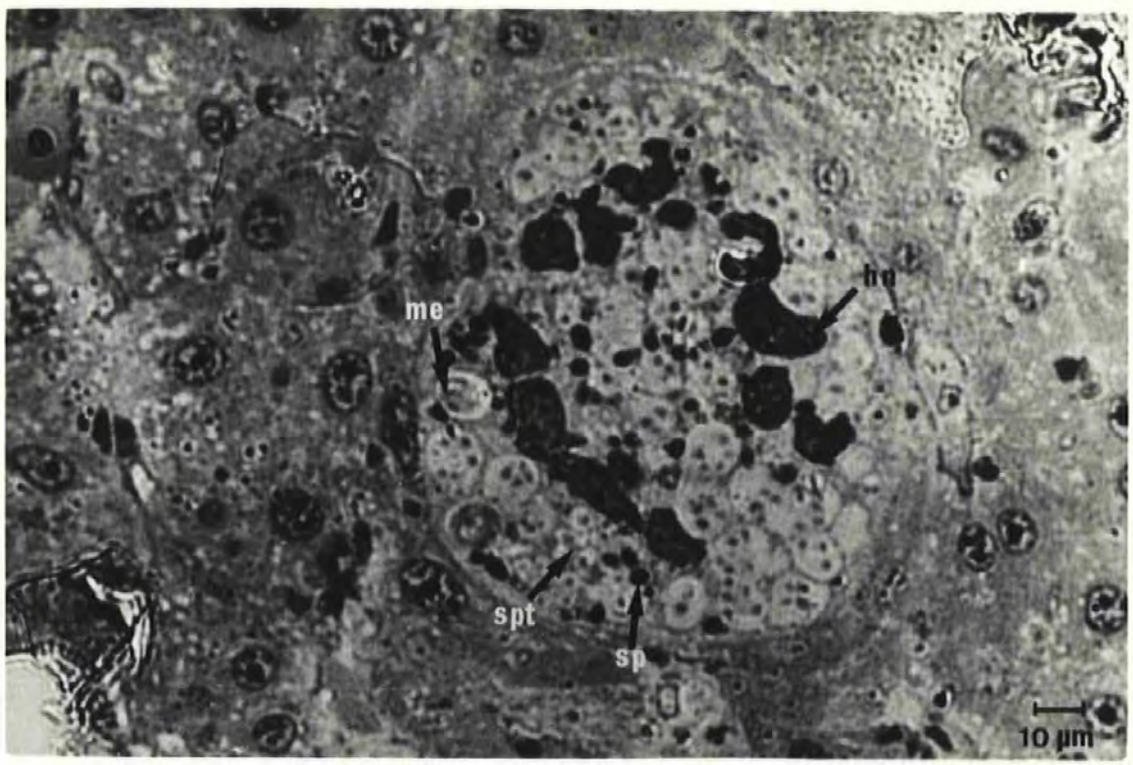
Figure 38 Early spore of Microgemma sp. from the liver of Taurulus bubalis showing a fully developed polar filament associated with the anchor disc. Note the small posterior vacuole.


mb
pl
n
pf
cy
e
0·5 µm


pv
n
pl
pf
ad
mb
0·5 µm

Figure 39 A methylene blue stained section of a young xenoma of Microgemma sp. from the liver of Taurulus bubalis. Note the reticulated host nucleus around the periphery of the cell.

Figure 40 A methylene blue stained section of a xenoma of Microgemma sp. from the liver of Taurulus bubalis showing the distribution of developmental stages. Note the small adjacent xenoma (arrowed).


me
hn
spt
sp
10 µm

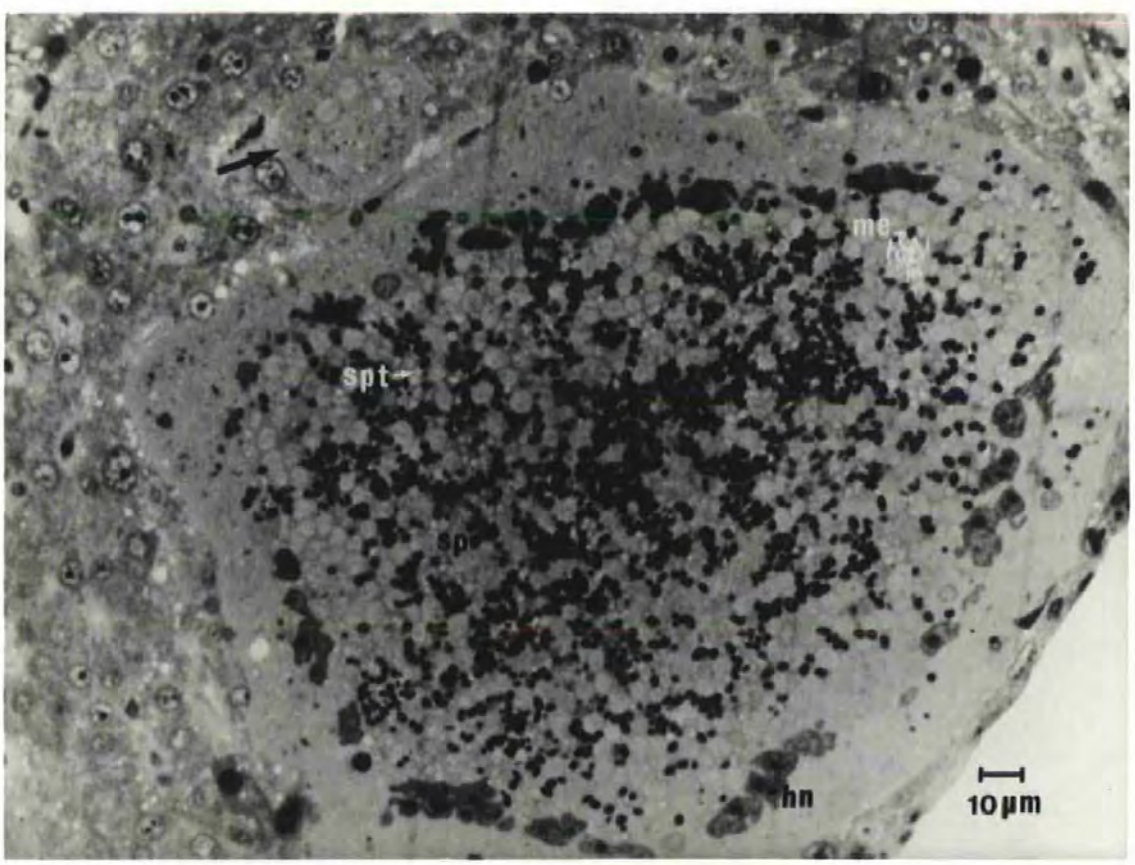
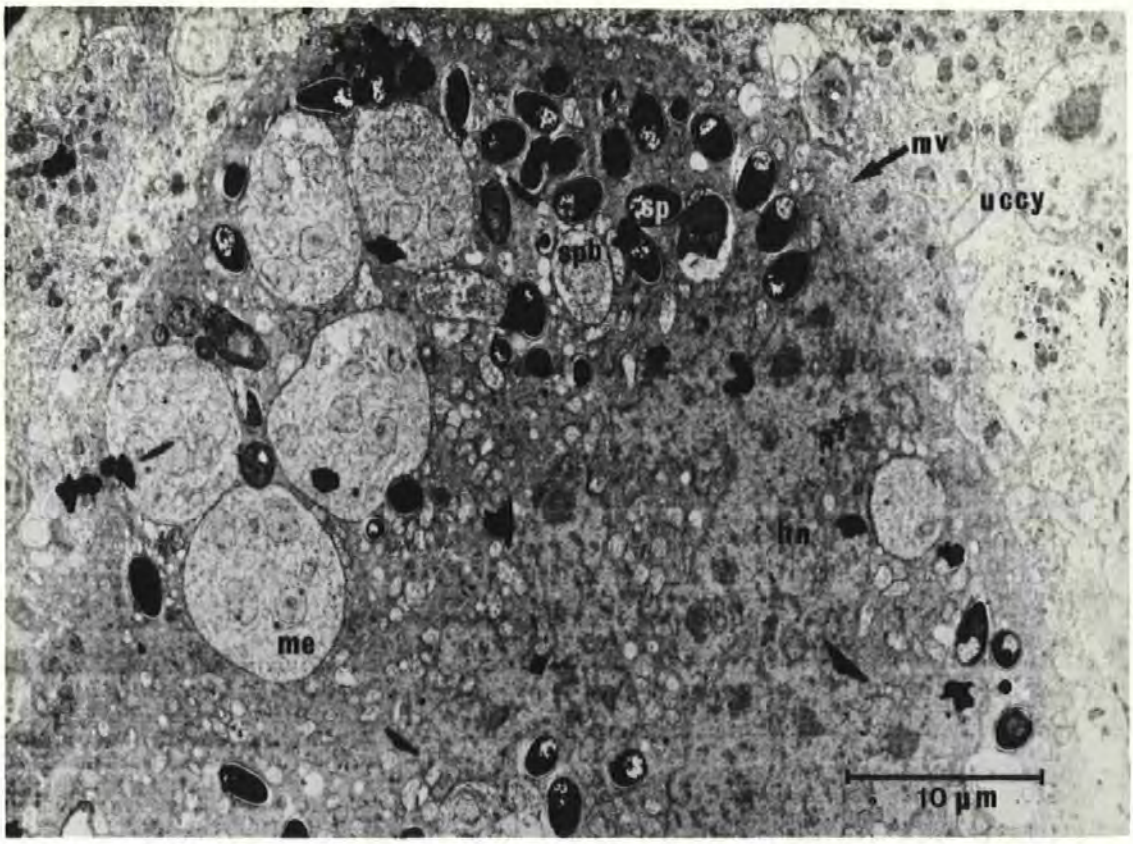

me
spt
sp
hn
10µm

Figure 41 Young xenomas of Microgemma sp. in the liver of Taurulus bubalis. Note the incomplete zonation of developmental stages.
(a) Note the microvilli (arrowed) suggesting the passage of nutrient from the adjacent cell.

Figure 41 (b) Further region of the same xenoma consisting of later developmental stages.


mv
uccy
sp
spb
hn
me
10 µm

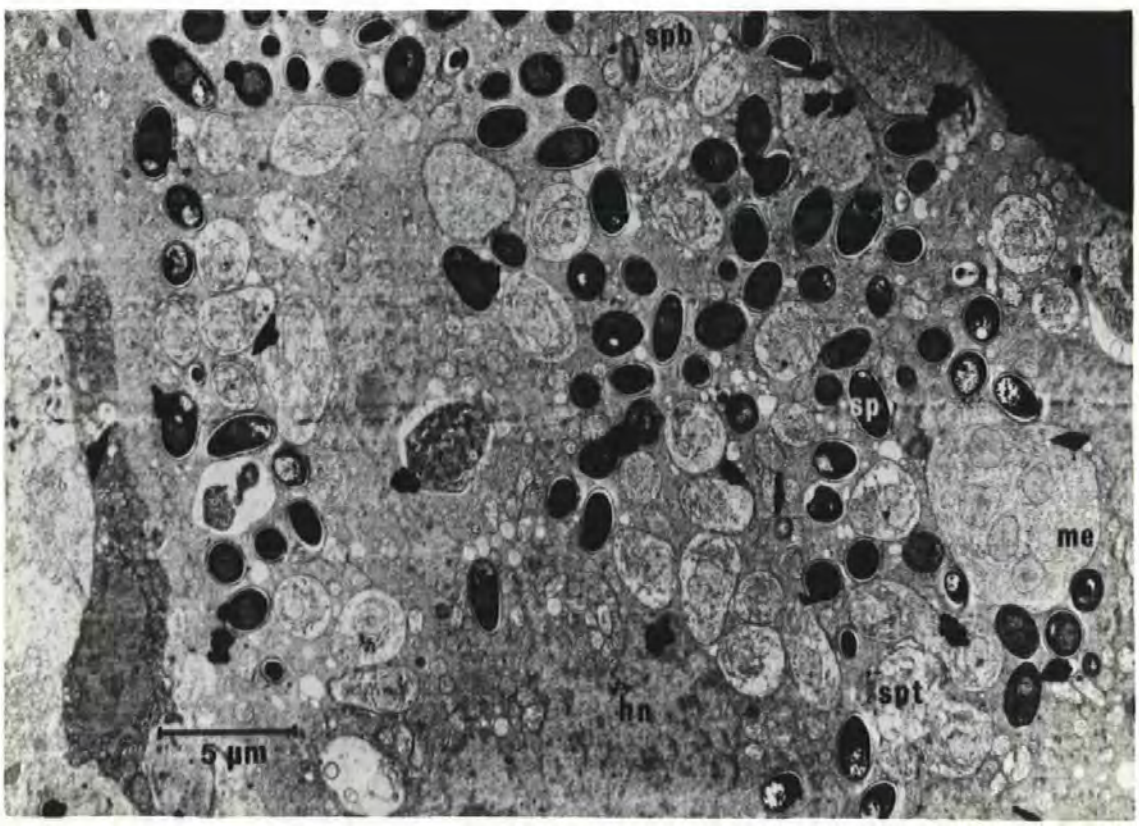
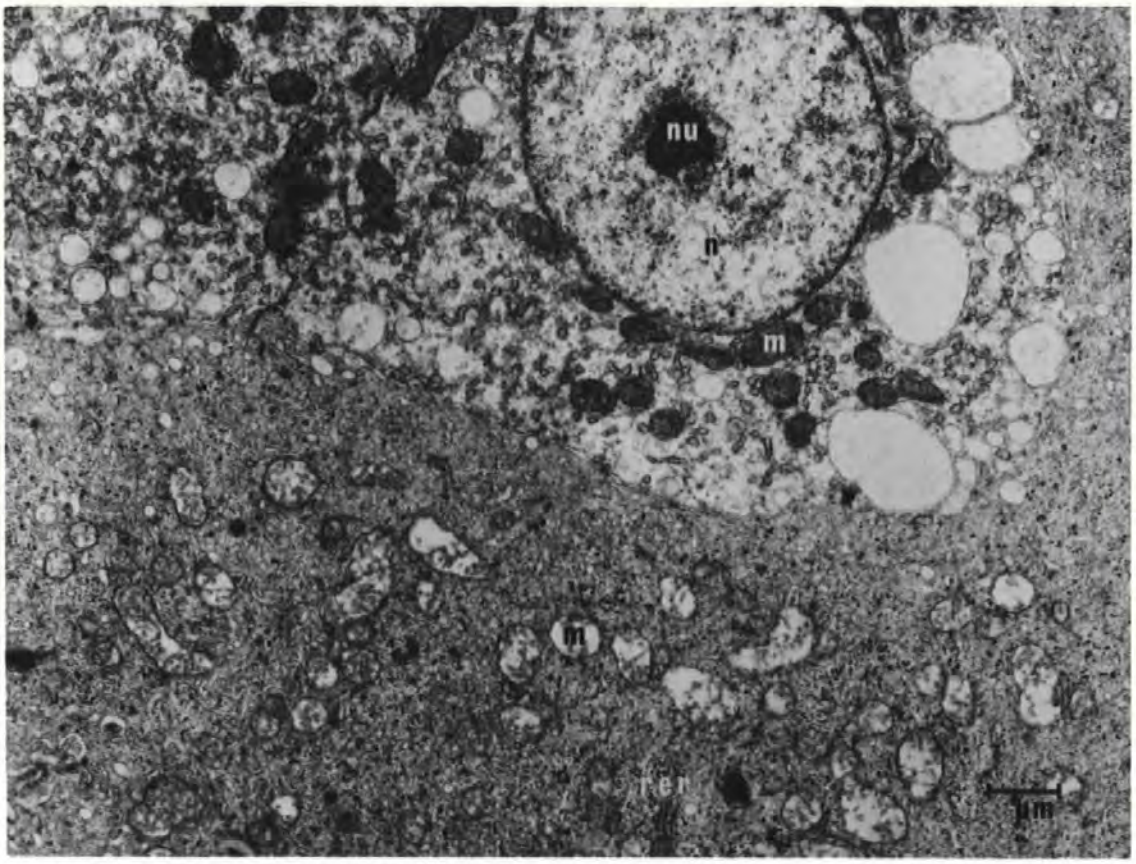

spb
sp
me
spt
hn
5 µm

Figure 42 To compare an uninfected liver cell with a cell infected with Microgemma sp.
(a) Note the greater density of cytoplasm in the infected cell and the pale mitochondria.

Figure 42 (b) A close up view of the boundary between infected and uninfected cells. Note the junctional complex.


nu
n
m
m
rer
µm

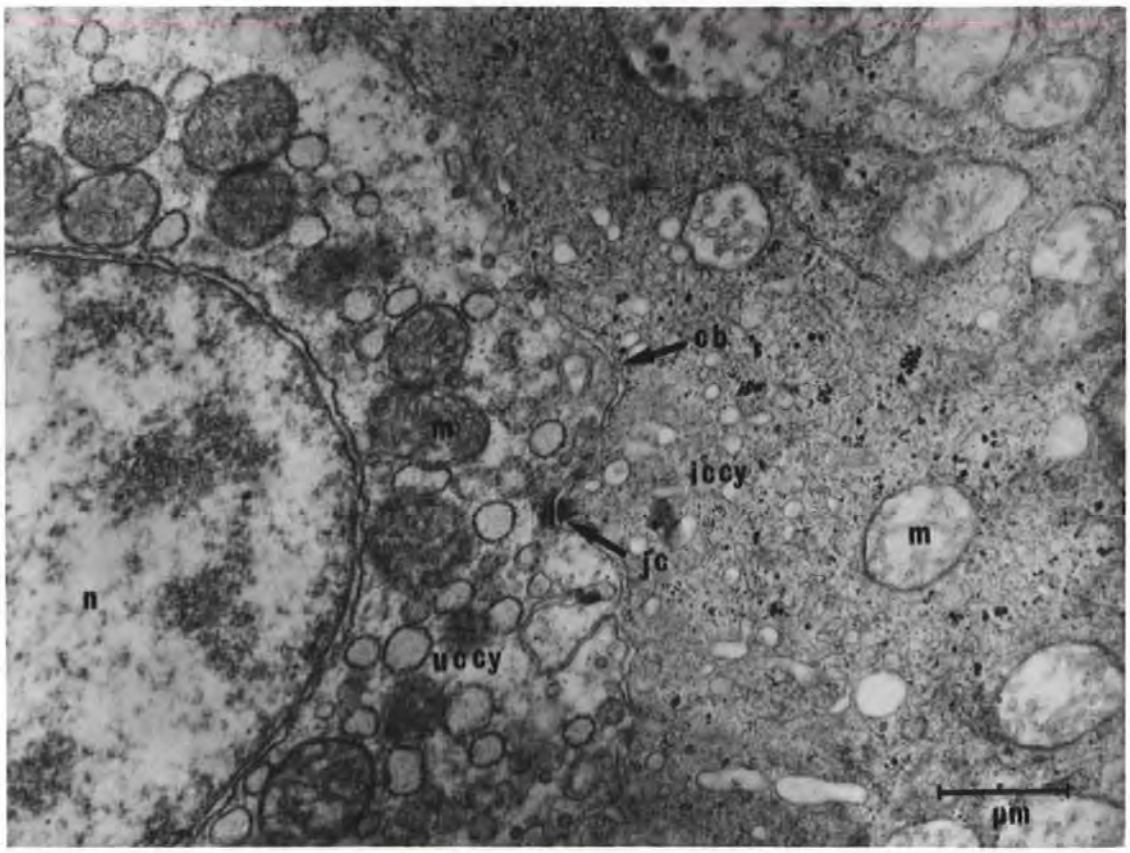
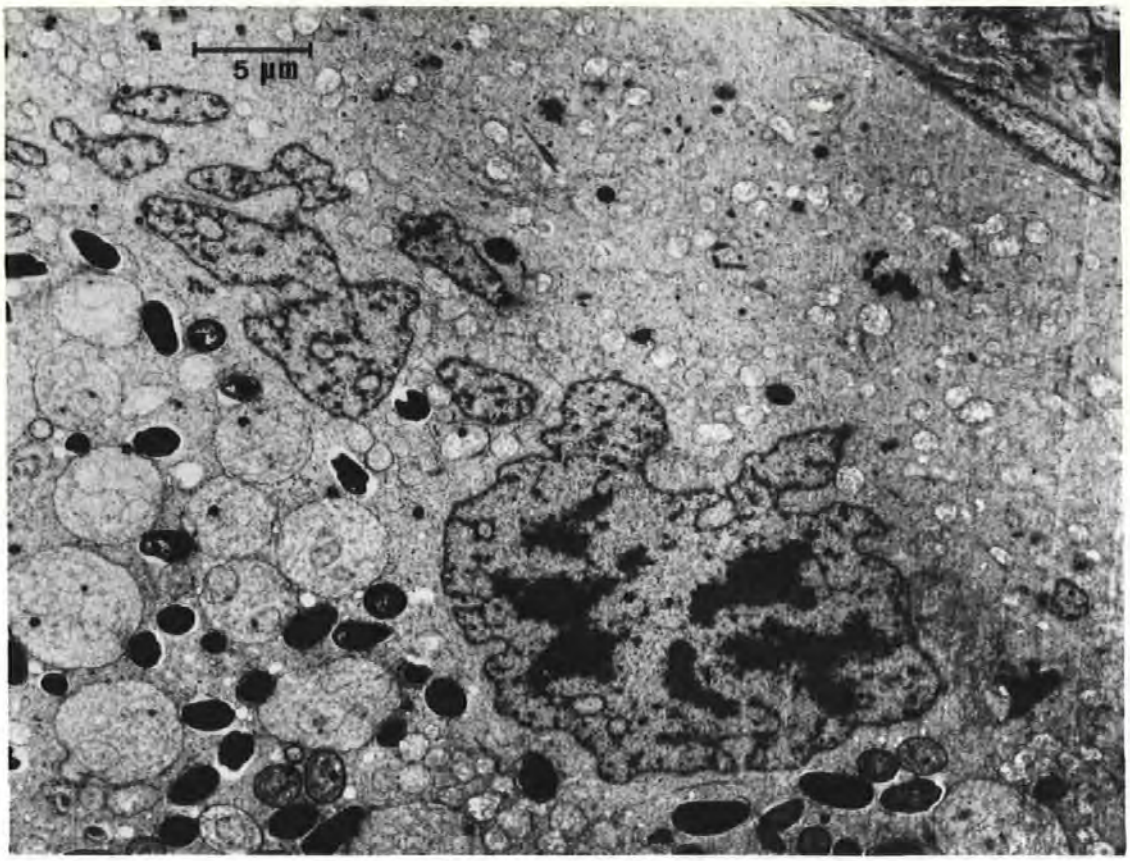

ob
m
iccy
m
jc
n
uccy
µm

Figure 43 Peripheral zone of xenoma of Microgemma sp. from the liver of Taurulus bubalis. Note the abundant mitochondria and ribosomes.

Figure 44 The second distinctive zone of the xenoma of Microgemma sp. from the liver of Taurulus bubalis. One reticulate host nuclei has distinct concentrations of chromatic material with mitochondria interspersed. Note the meronts.


5 µm

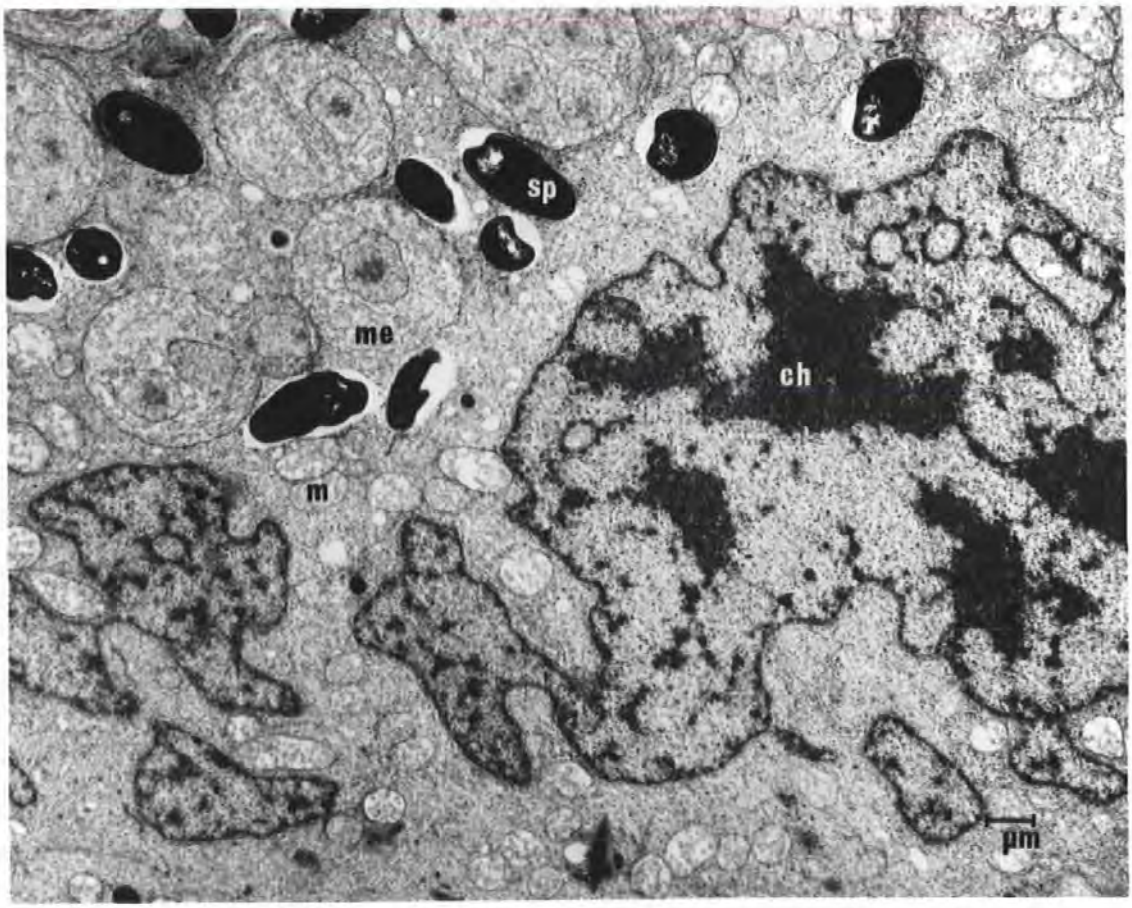
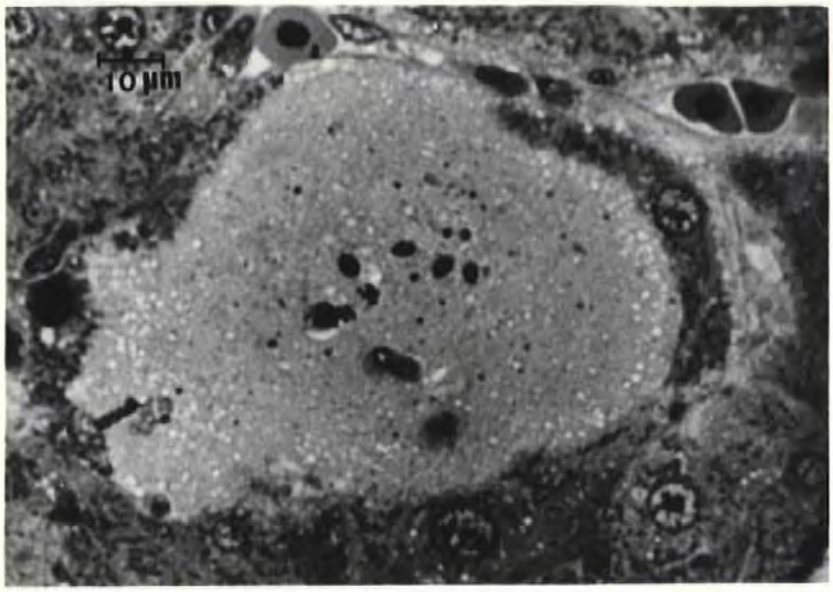
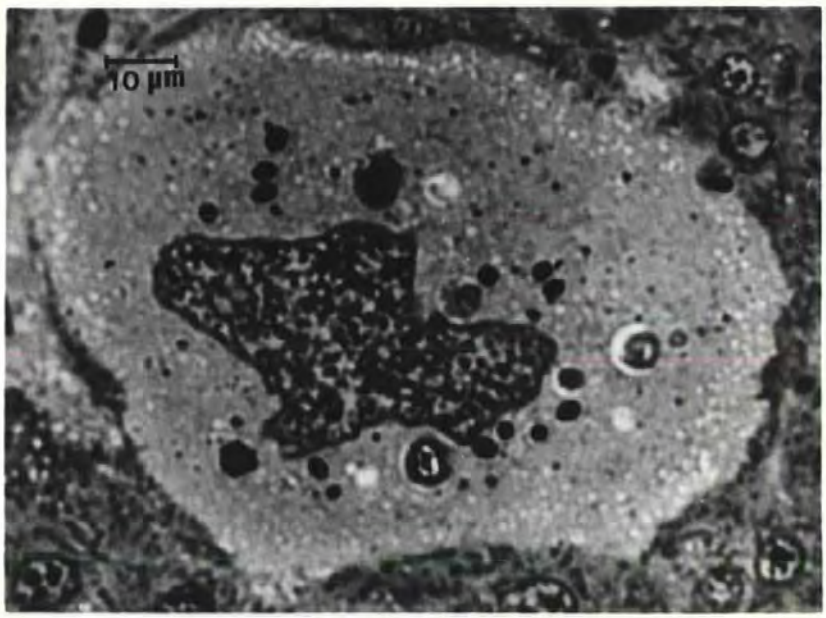

sp
me
m
ch
µm

Figure 45 Methylene blue stained sections to show early invasion of liver cell in Taurulus bubalis by Microgemma sp.
(a) Note the hypertrophy of the cell and concentration of spores at the centre.

Figure 45 (b) Note the enlarged nucleus with surrounding developing parasitic stages.

Figure 45 (c) Note the apparent fragmentation of nucleus and its migration towards the periphery of the cell.


10 µm


10 µm

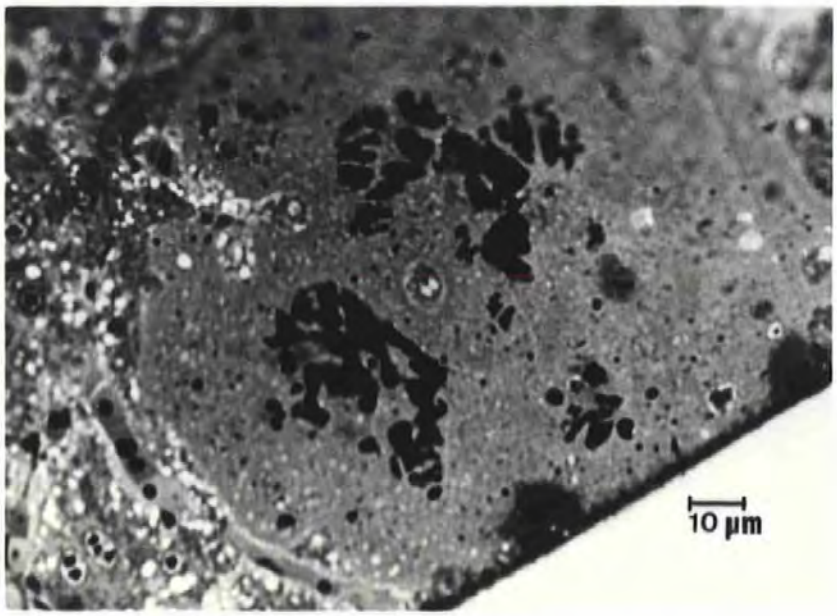
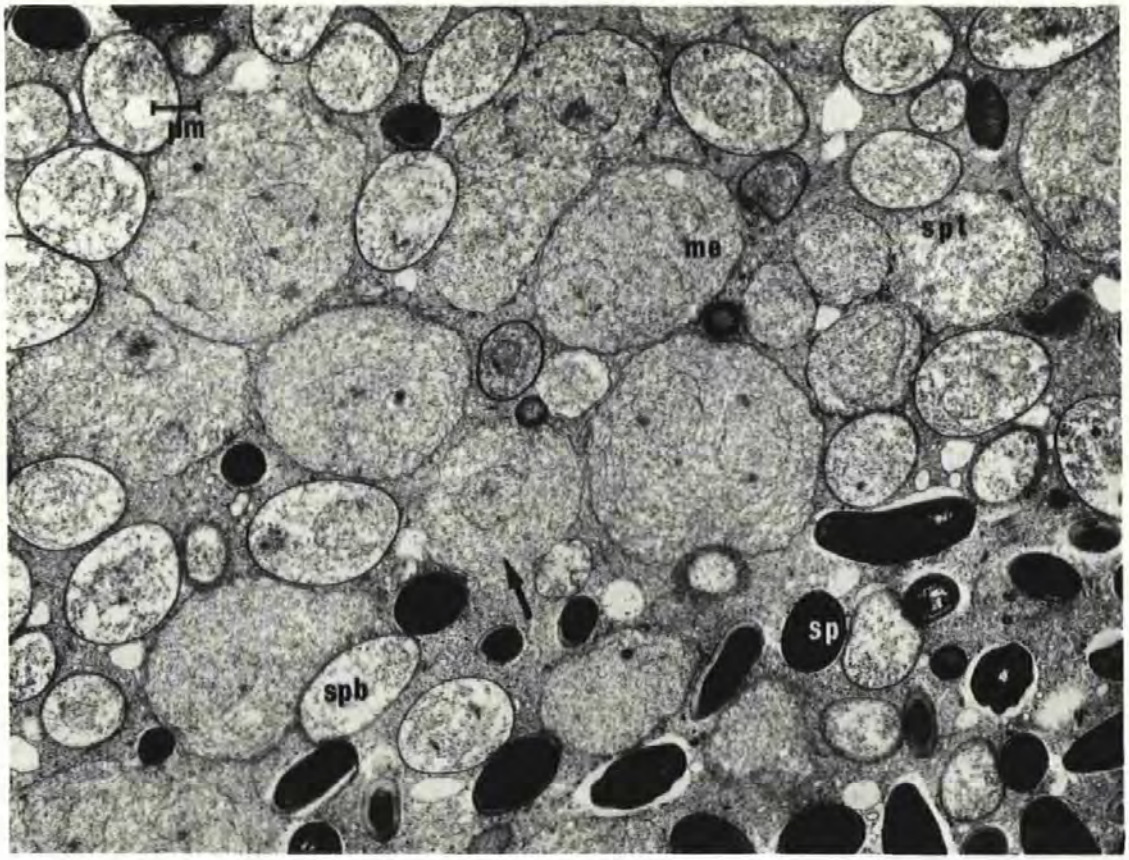

10 µm

Figure 46 The third zone of the xenoma of Microgemma sp. from the liver of Taurulus bubalis. Note the abundant sporogonial stages interspersed with meronts and the occasional sporoblast and spores.

Figure 47 The centre of the xenoma of Microgemma sp. from the liver of Taurulus bubalis. Spores and sporoblasts almost exclusively occupy this region.


µm
me
spt
sp
spb

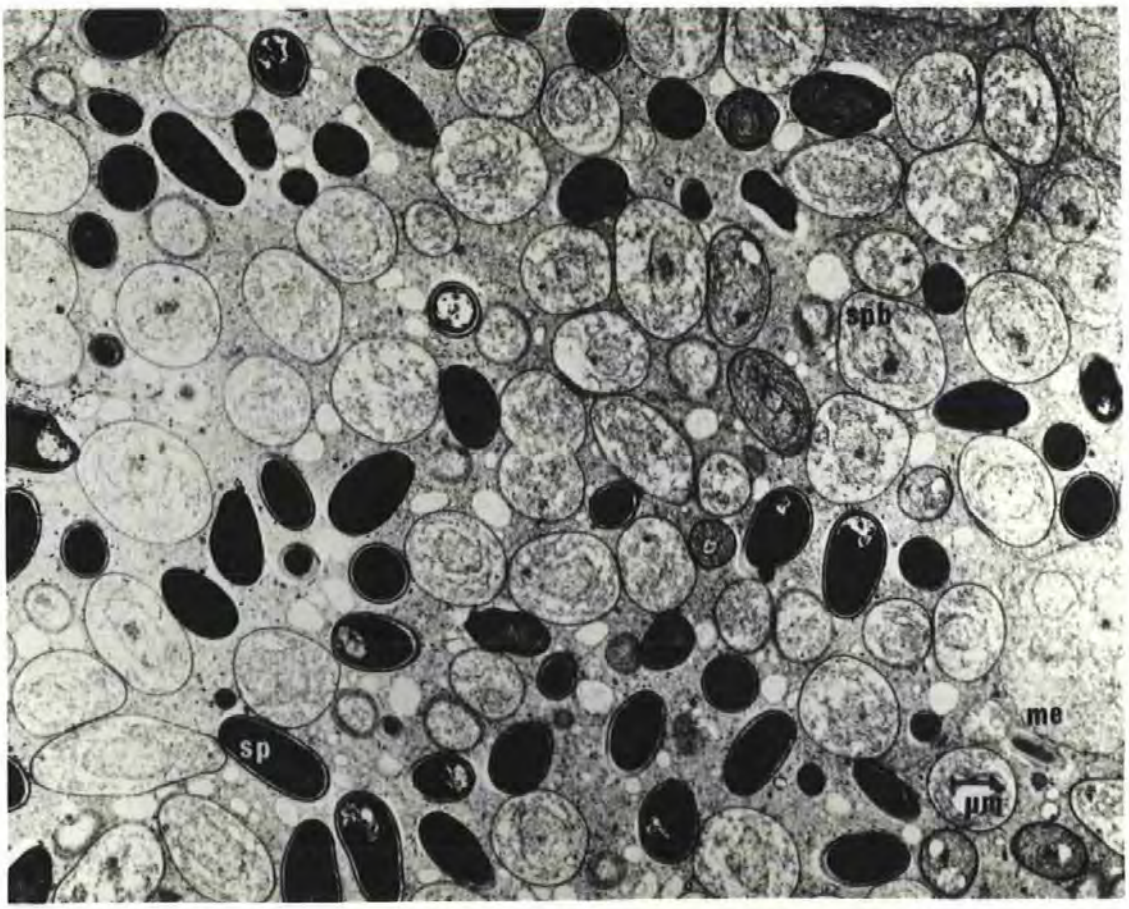
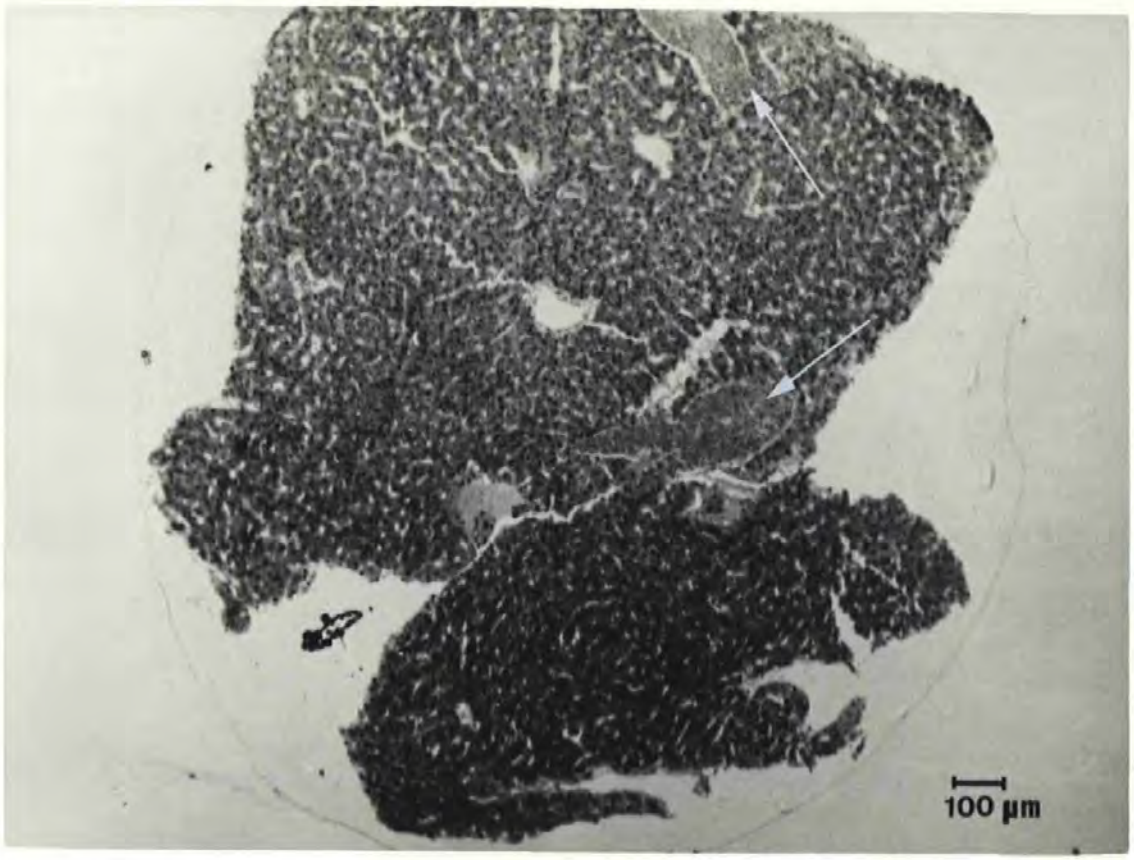

spb
sp
me
µm

Figure 48 Section of liver from Taurulus bubalis at microscope level.
(a) Low power view. Note the irregular shape of xenomas (arrowed).

Figure 48 (b) High power view of section. Note the close proximity of the xenoma to the sinusoid and that the spores have been phagocytosed.


100 µm

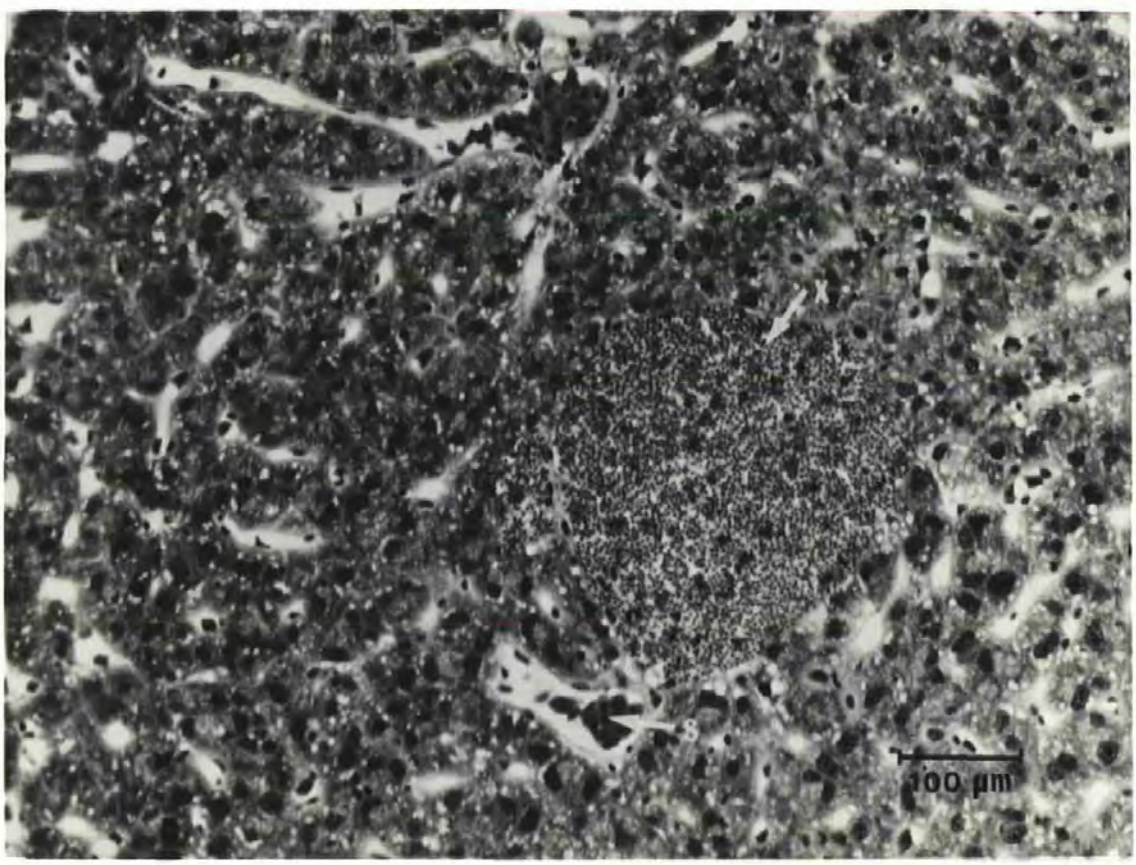
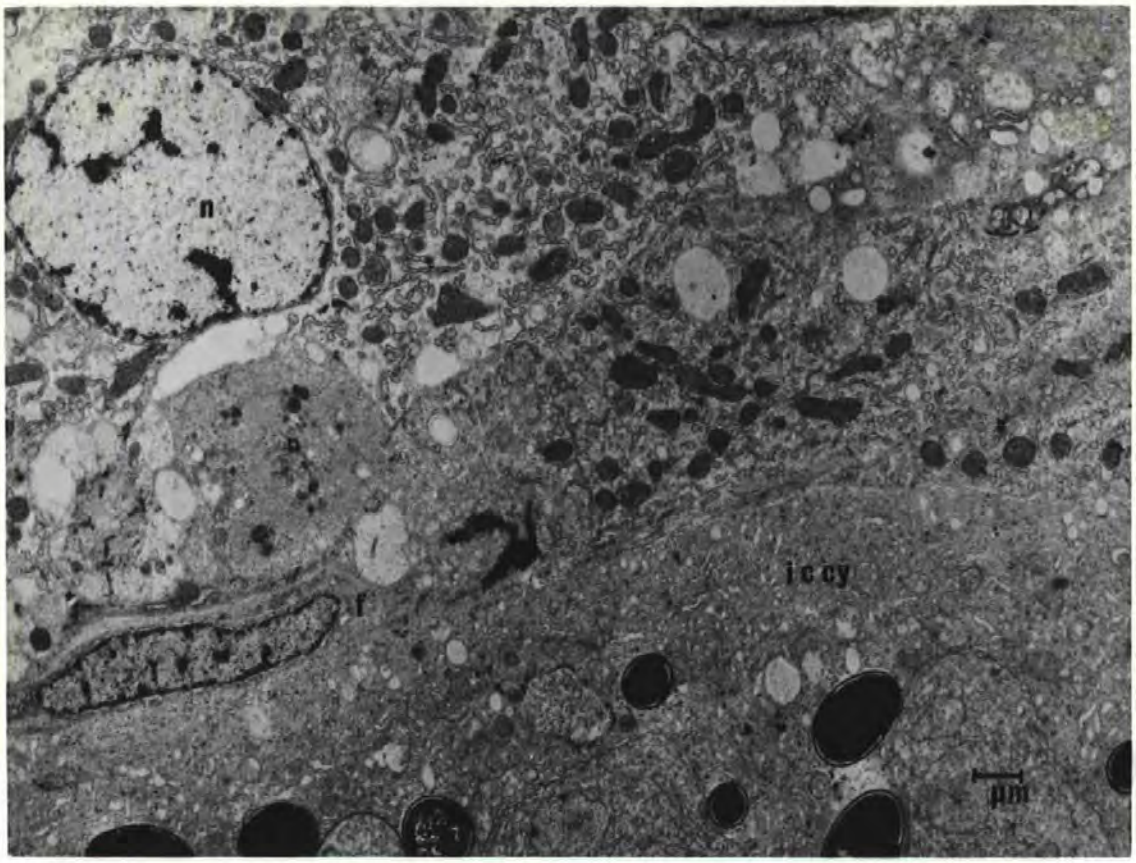

100 µm

Figure 49 Initial stages of isolation of xenoma of Microgemma sp. from the liver cells of Taurulus bubalis by fibroblast cells.

Figure 50 Isolation of the xenoma of Microgemma sp. Note in addition to the walling off of the xenoma by fibroblast cells, the deposition of collagin.


n
f
i c cy
µm

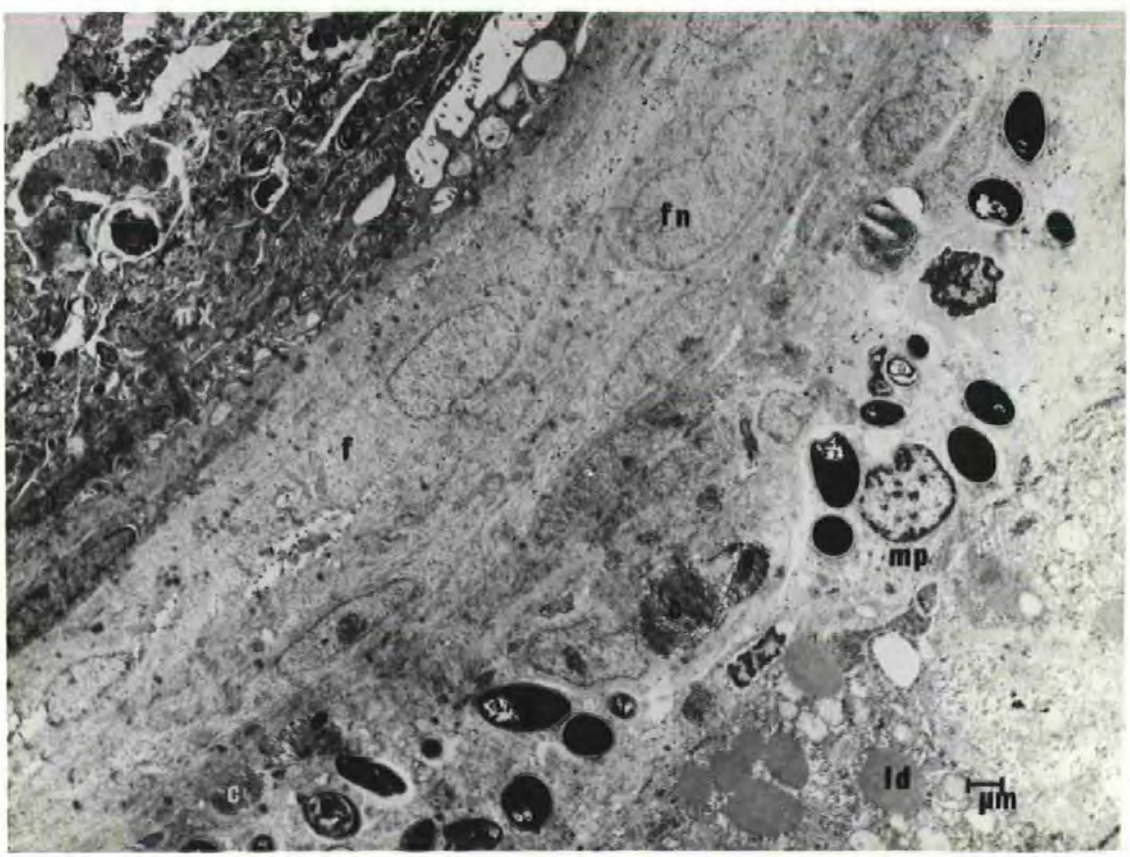
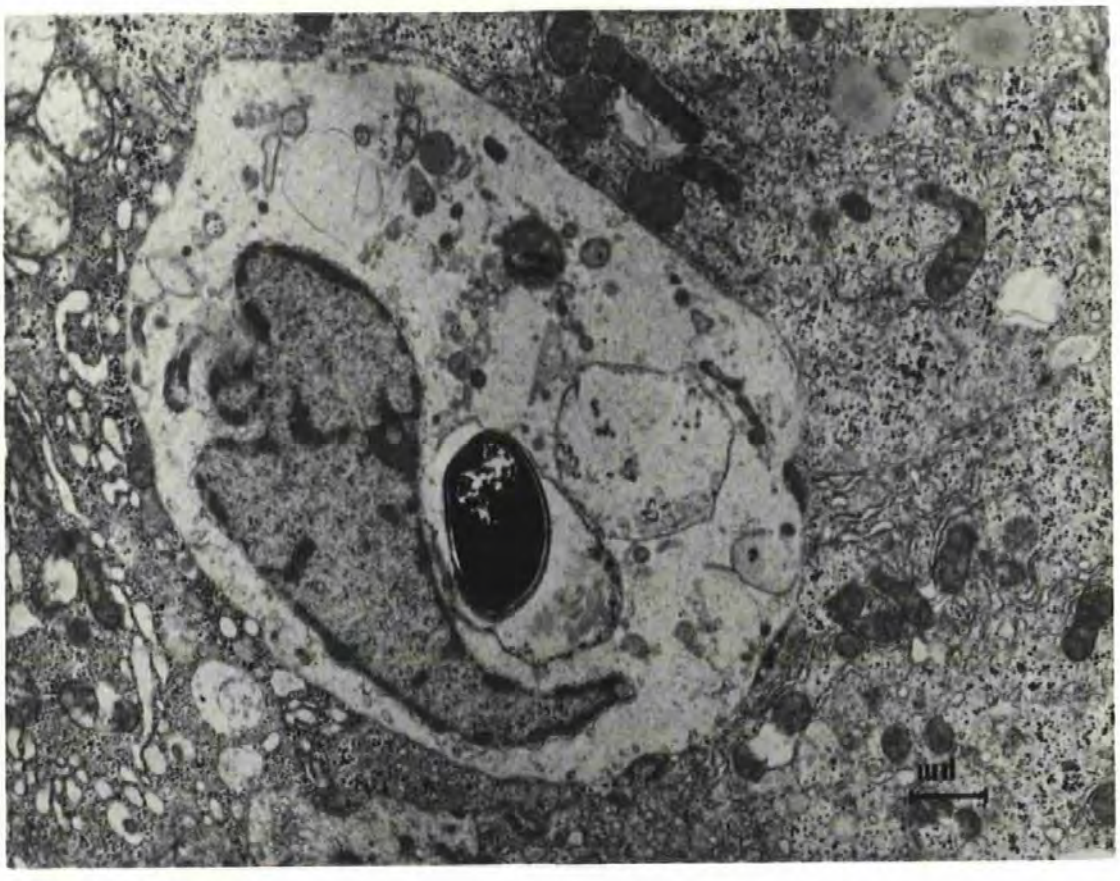

fn
mx
f
mp
c
ld
µm

Figure 51 Phagocytes of Taurulus bubalis engulfing spores of Microgemma sp. released from the xenoma.

Figure 52 To show spore of Microgemma sp. engulfed by macrophage of Taurulus bubalis.



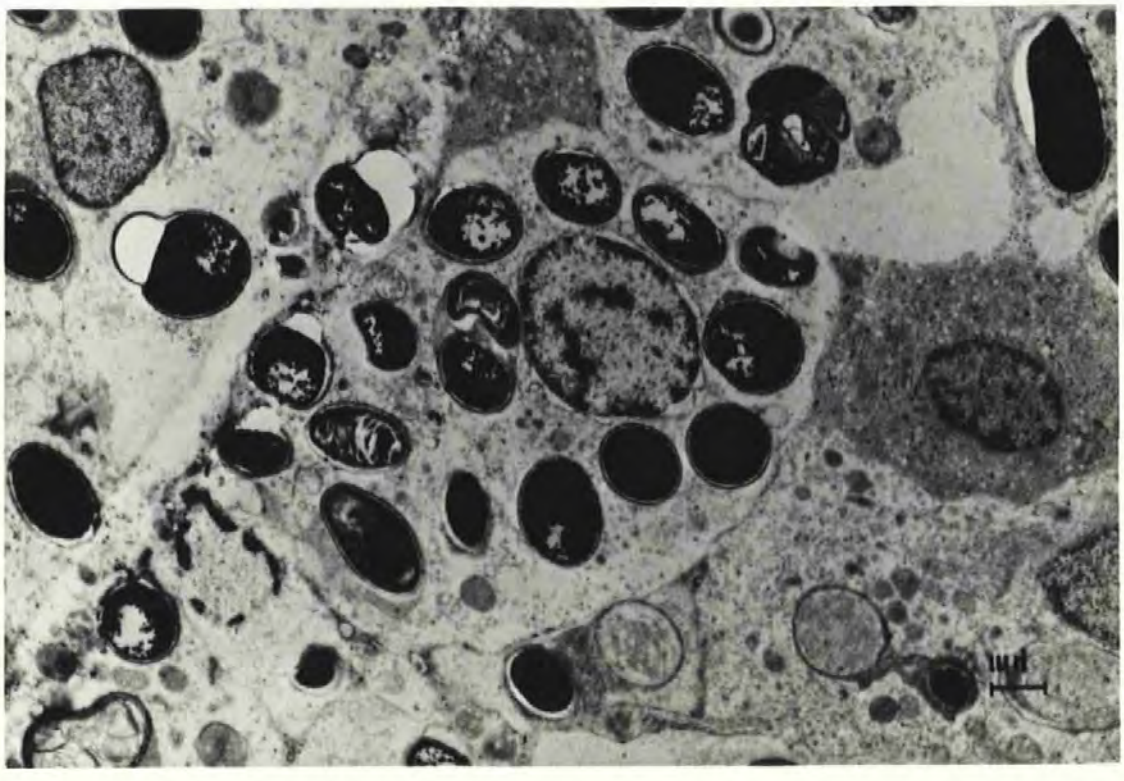
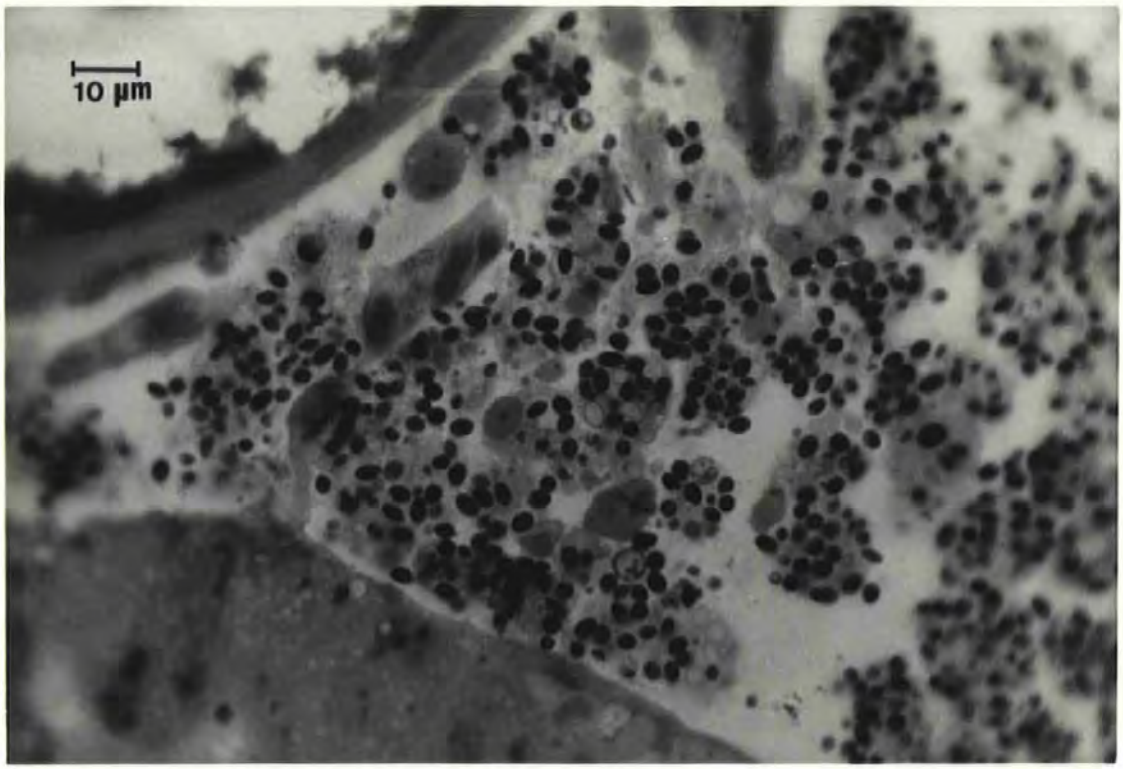


Figure 53 Macrophages in blood vessel of Taurulus bubalis, liver full of spores from degraded xenoma.

Figure 54 Xenomas of Microgemma sp. in the liver of Taurulus bubalis at various stages of degradation. X_1 shows spores free in the xenoma, X_2 shows spores that have been phagocytosed.


10 µm

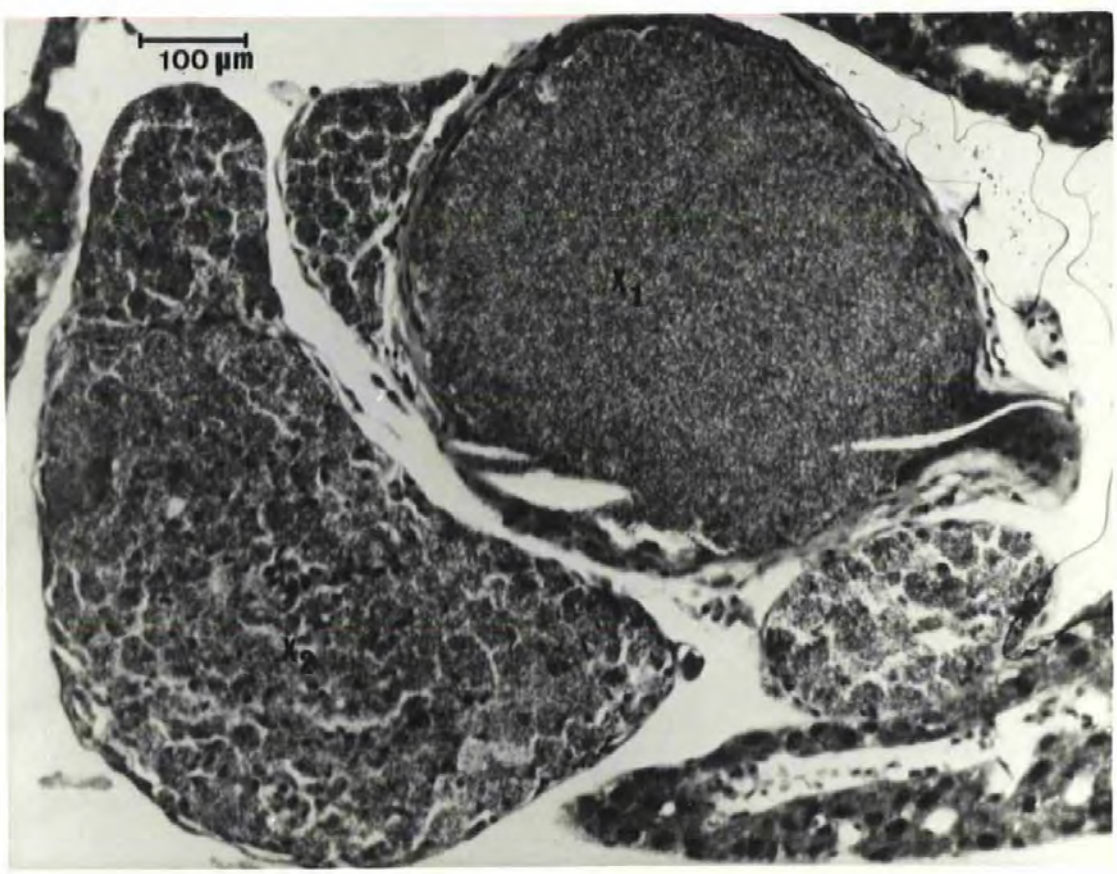
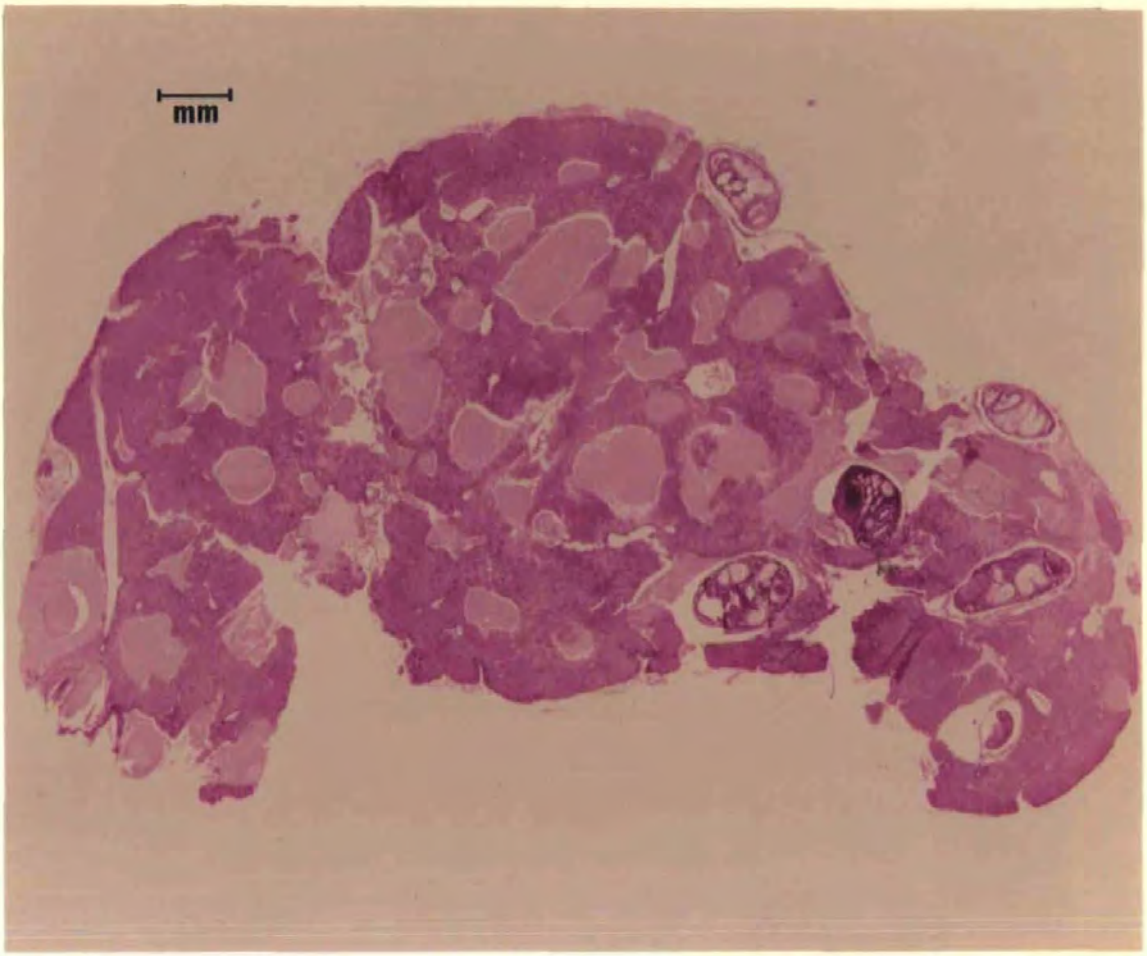

100 µm
X1
X2

Figure 55 Section of liver from Taurulus bubalis stained with Periodic Acid - Schiff. The dark pink areas indicate the presence of polysaccharides which a diastase control showed here to be glycogen. Note the absence of polysaccharide in the xenomas.

Figure 56 Section of liver from Taurulus bubalis stained with Mallory's Trichrome. Blue areas indicate the presence of connective tissue such as collagen, while pink indicate cytoplasmic material and red the nuclei.


mm

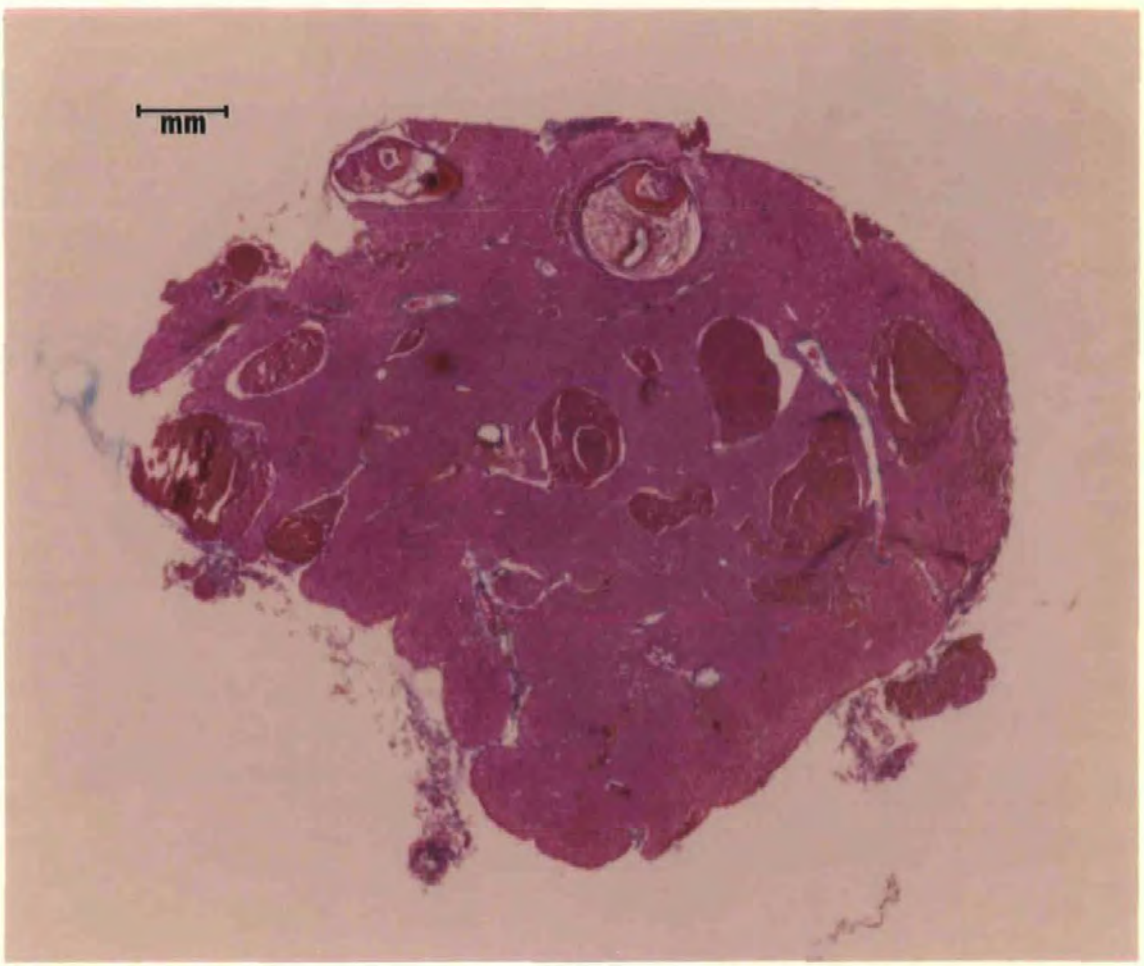

mm

Figure 57 Hepatic microsporidian spores from various inshore fish hosts. All markers represent 0.5 um.

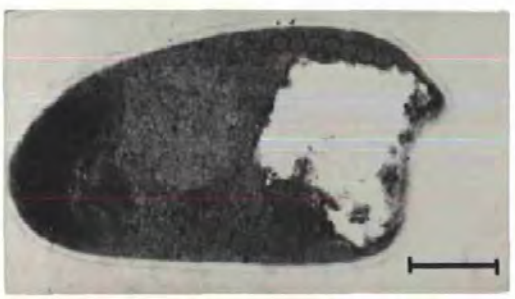
(a) Spore from Ciliata mustela. Note the 6 - 7 coils of polar filament and inclusion body.

(b) Spore from Gaidropsarus mediterraneous again with 6 - 7 coils of polar filament.

(c) and (d) Spores from Scopthalmus maximus with 6 coils of polar filament and inclusion body.

(e) Spore from Taurulus bubalis for comparison. Note the 6 - 8 coils of polar filament.

(f) Spore from Crenilabrus melops. Note the inclusion body, small posterior vacuole and prominent nuclear membrane.



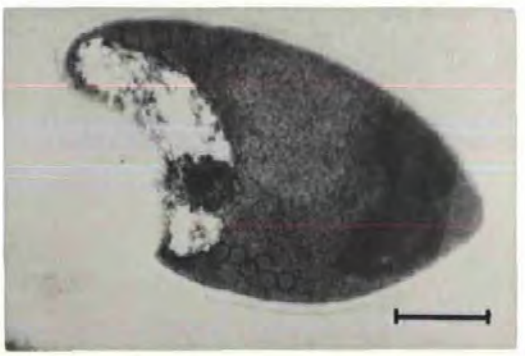


Figure 58 Intramuscular infection of Cottus gobio with Pleistophora vermiformis (Leger 1904). Note all developmental stages present within pansporoblastic membranes.

Figure 59 Intramuscular species of Pleistophora sp. of Taurulus bubalis. Note all developmental stages within pansporoblastic membranes.


2µm

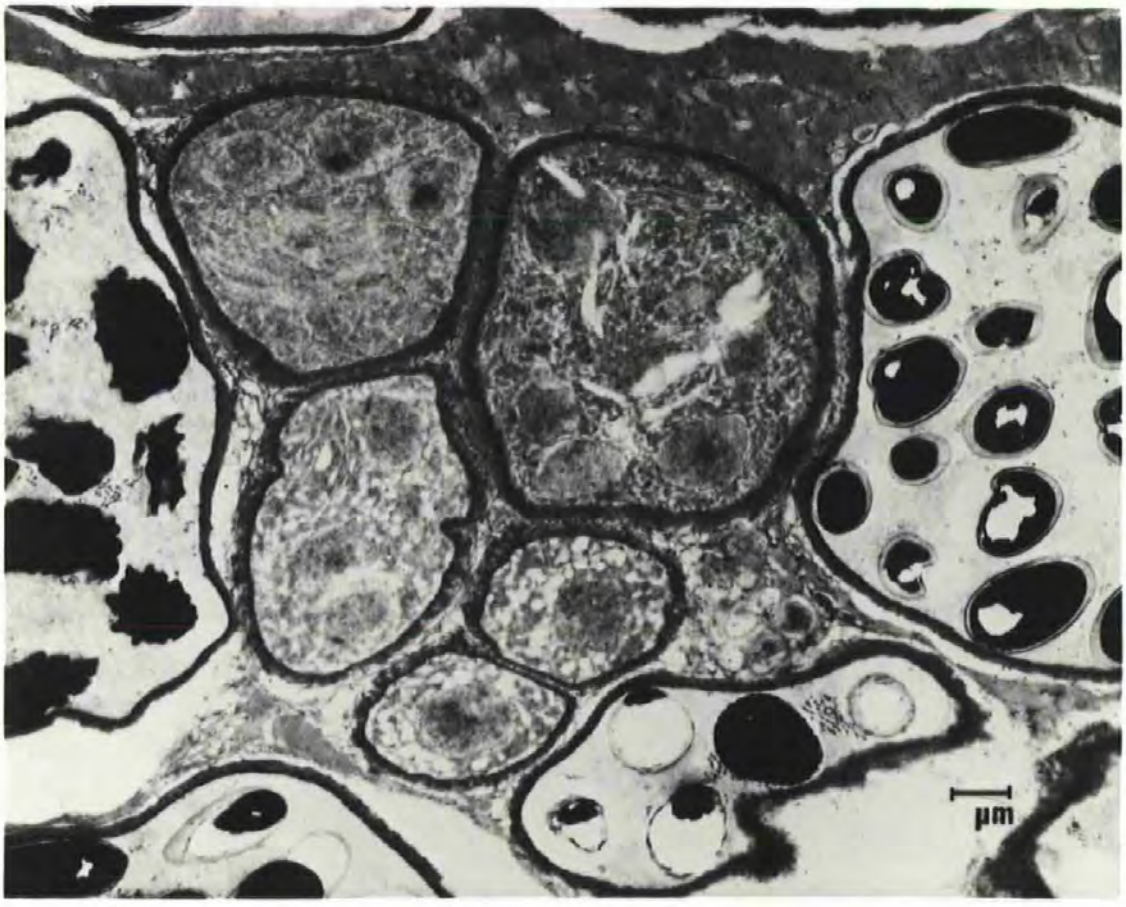

1µm

Figure 60 Merogonial stages of Pleistophora sp. in the musculature of Taurulus bubalis.

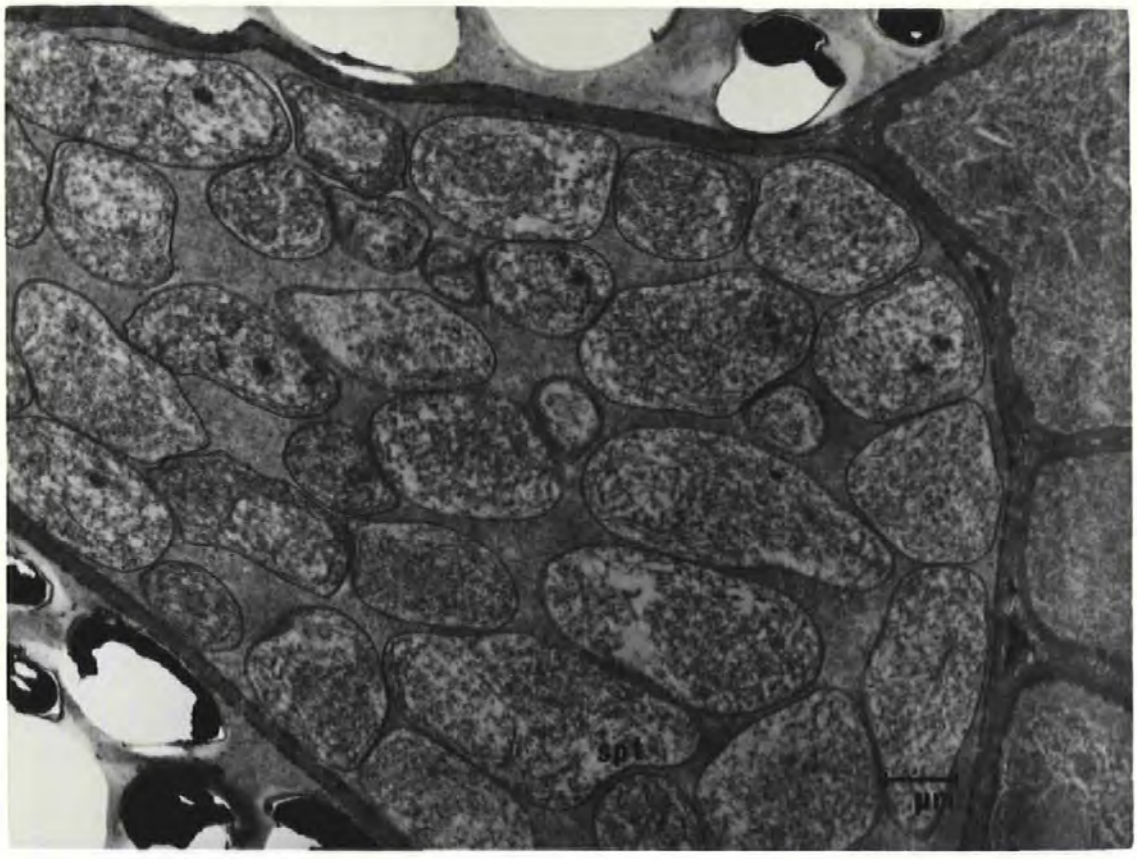
Figure 61 Anterior region of spore of Pleistophora sp. from the musculature of Taurulus bubalis. Note the anchor disc and well defined polaroplast.


µm


0·5 µm

Figure 62 Sporogonic stages of Pleistophora vermiformis from the musculature of Cottus gobio.

Figure 63 Sporogonic stages of Pleistophora sp. from the musculature of Taurulus bubalis. Note the microtubules in the pansporoblastic cytoplasm.


spt
μm

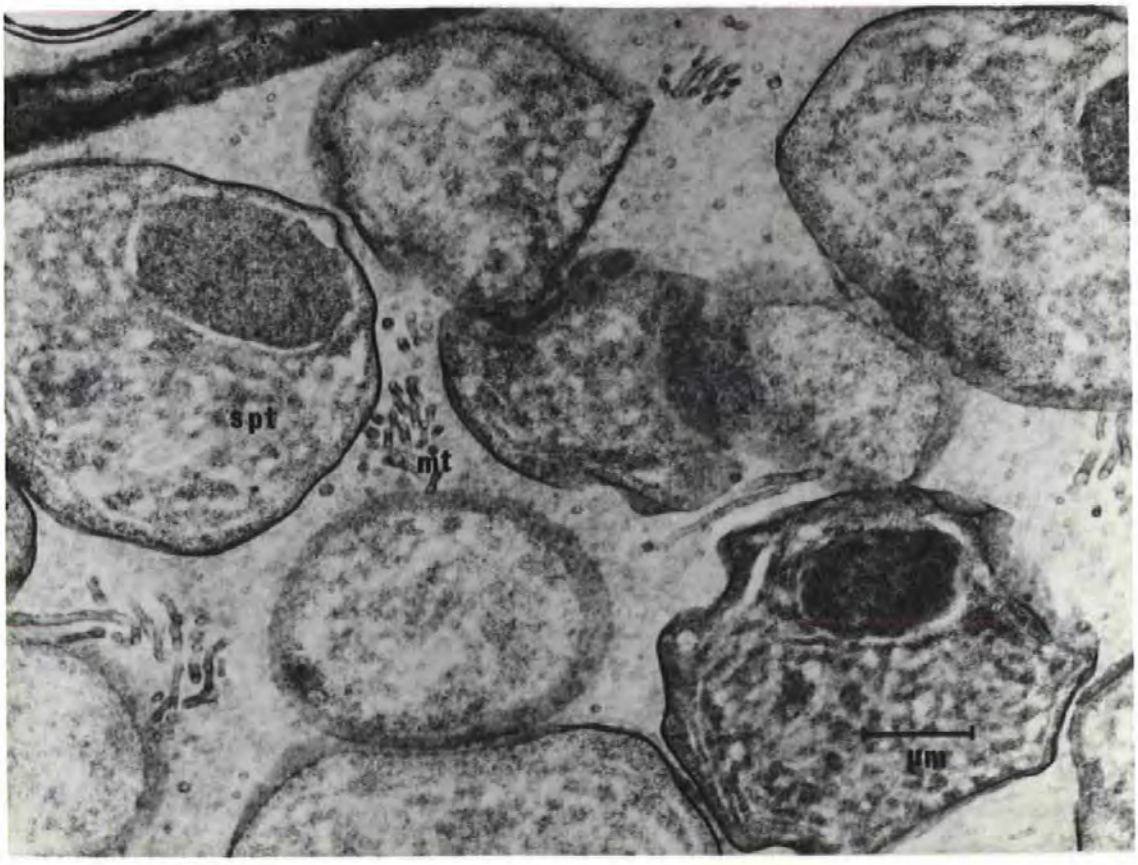
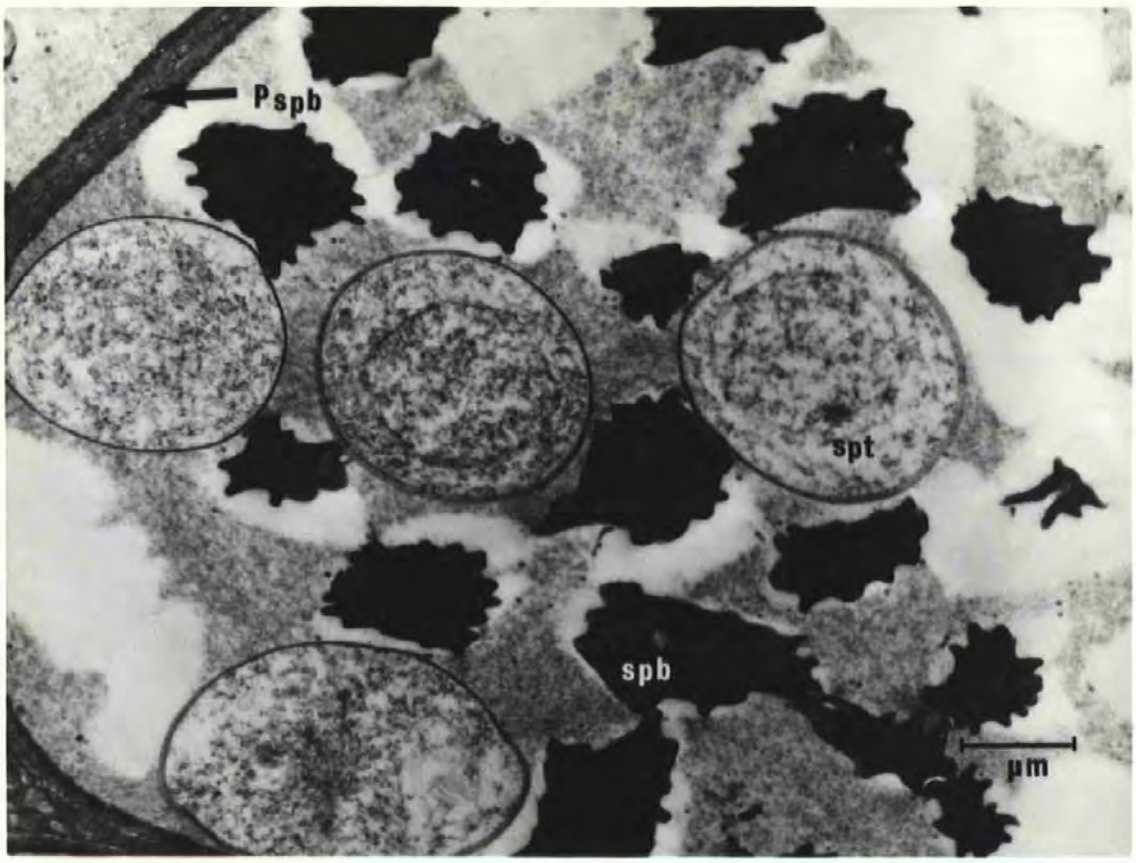

spt
mt
μm

Figure 64 *Pleistophora vermiformis* from the musculature of *Cottus gobio* to show sporogony. Note the stellate nature of the sporoblast.

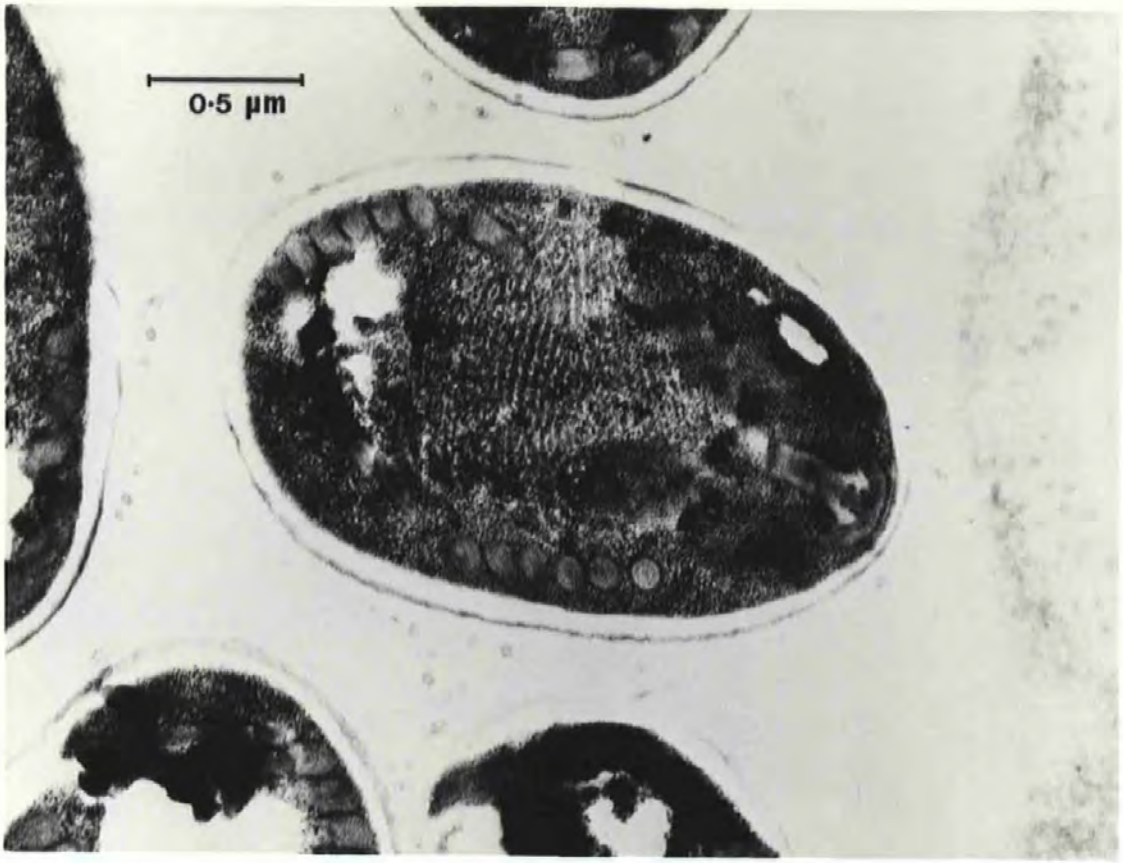
Figure 65 Details of pansporoblastic membrane of *Pleistophora* sp. from the musculature of *Taurulus bubalis*. Note the mitochondria in the adjacent muscle cells.


Pspb
spt
spb
µm


P spb
0·5µm

Figure 66 Mature spore of *Pleistophora* sp. from the musculature of *Taurulus bubalis*. Note the 6 coils of polar filament.

Figure 67 Mature spore of *Pleistophora vermiformis* from *Cottus gobio*. Note the 15 coils of polar filament.


0·5 µm


0·5 µm

Figure 68 To show otoliths of Taurulus bubalis from each age group collected in summer and autumn.

(a) Otolith from group 'O' fish collected in July

(b) Otolith from group 'I' fish collected in July

(c) Otolith from group 'II' fish collected in July

(d) Otolith from group 'O' fish collected in October

(e) Otolith from group 'I' fish collected in October

(f) Otolith from group 'II' fish collected in October

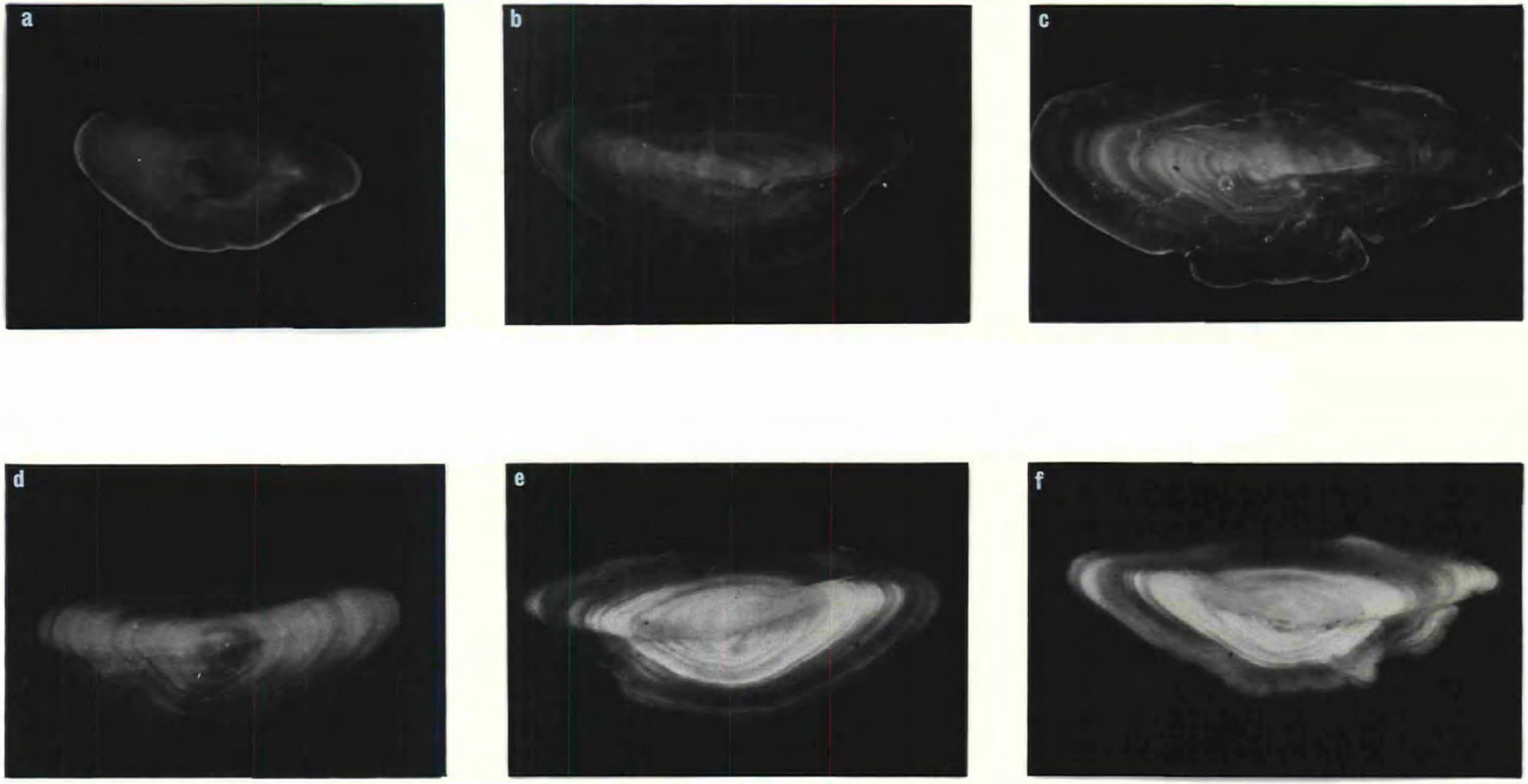

a
b
c
d
e
f

Fig 69 To show otoliths of Taurulus bubalis from each age group collected in winter and spring.

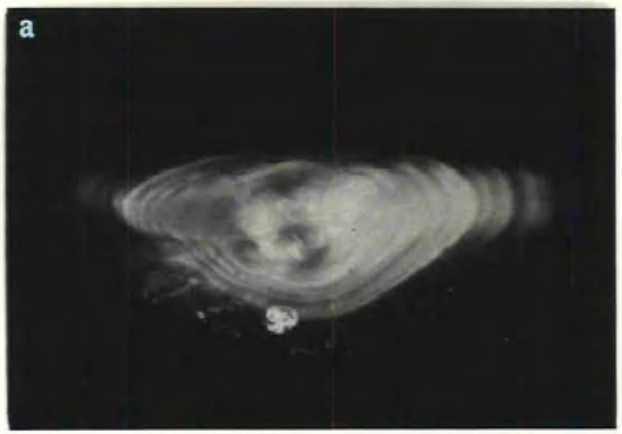
(a) Otolith from group 'O' fish collected in February

(b) Otolith from group 'I' fish collected in February

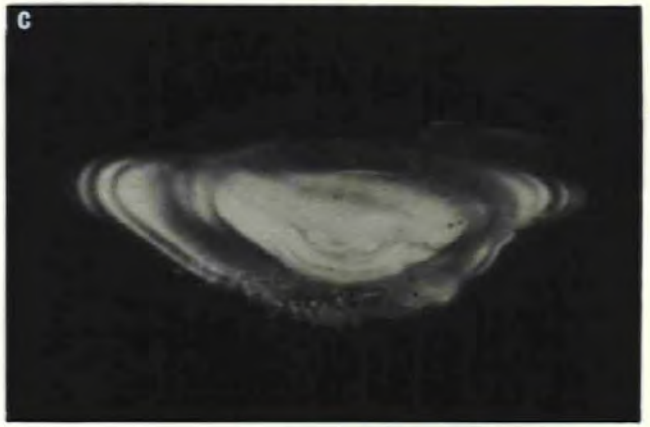
(c) Otolith from group 'II' fish collected in February

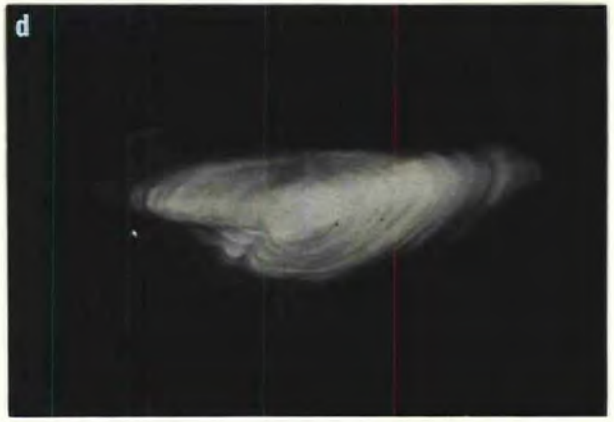
(d) Otolith from group 'I' fish collected in April

(e) Otolith from group 'II' fish collected in April


a


b


c


d

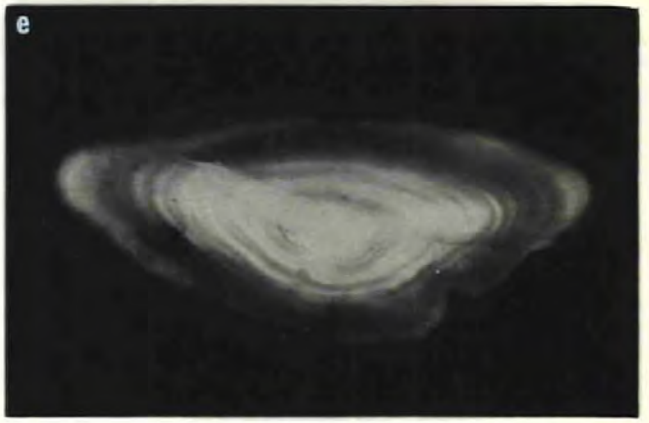

e

Figure 70 Seasonal length/frequency variation in T.bubalis from Portwrinkle, together with the mean length of each age group calculated from otoliths.

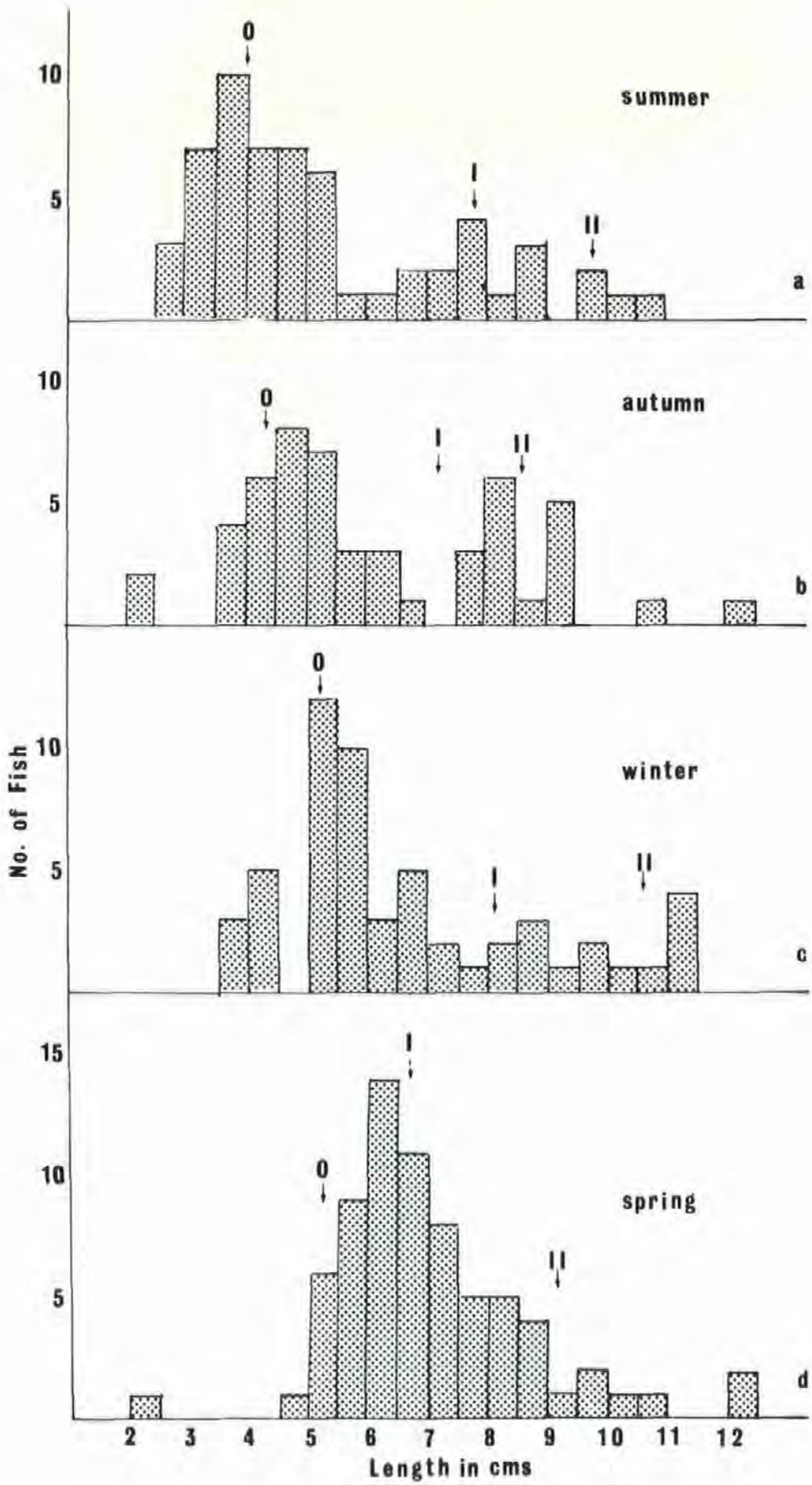
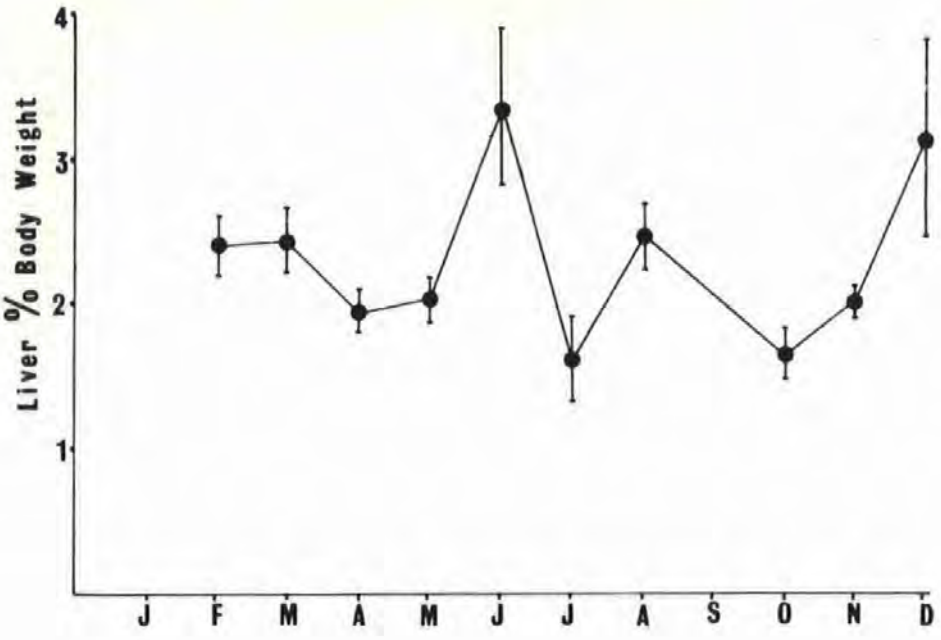

0
I
II
summer
a
10
5
autumn
b
winter
c
15
spring
d
No. of Fish
2
3
4
5
6
7
8
9
10
11
12
Length in cms

Figure 71 Monthly variation of mean liver percentage body weight in T.bubalis collected from Portwrinkle.

Figure 72 Monthly variation of mean condition factor in T.bubalis collected from Portwrinkle.


Liver % Body Weight
4
3
2
1
J
F
M
A
M
J
J
A
S
O
N
D

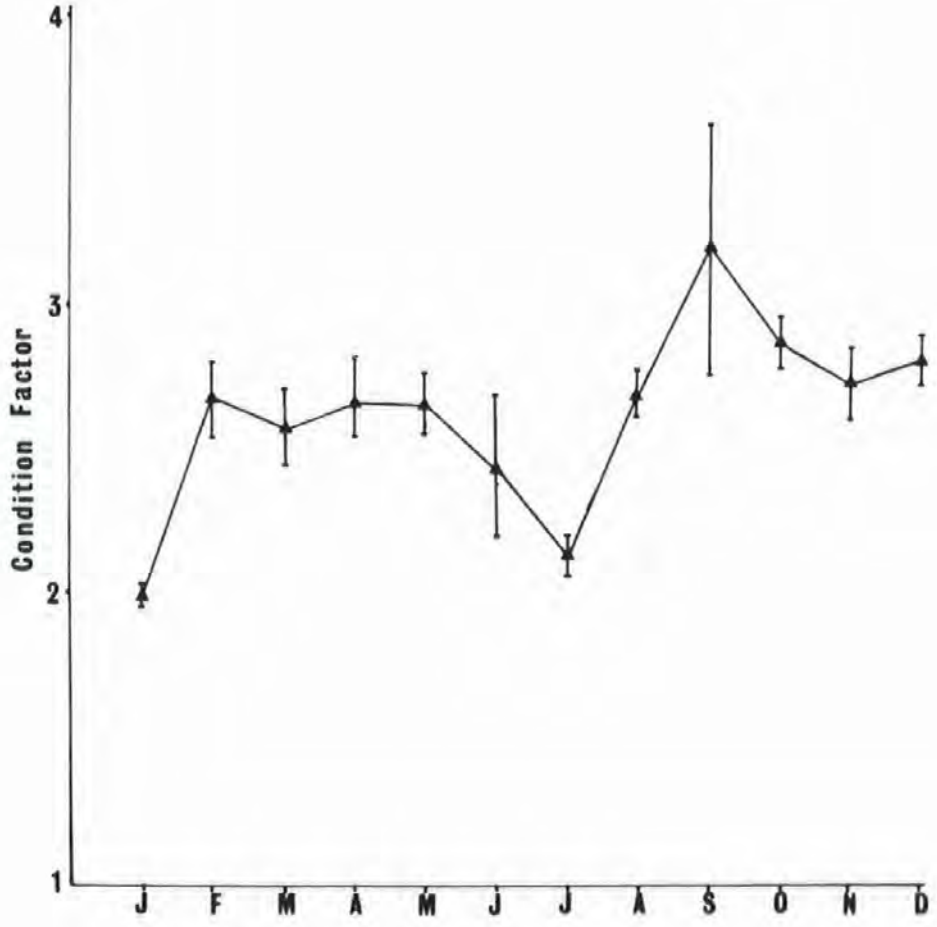

Condition Factor
4
3
2
1
J
F
M
A
M
J
J
A
S
O
N
D

Figure 73 Monthly variation of mean lipid percentage liver weight in *T.bubalis* collected from Portwrinkle.

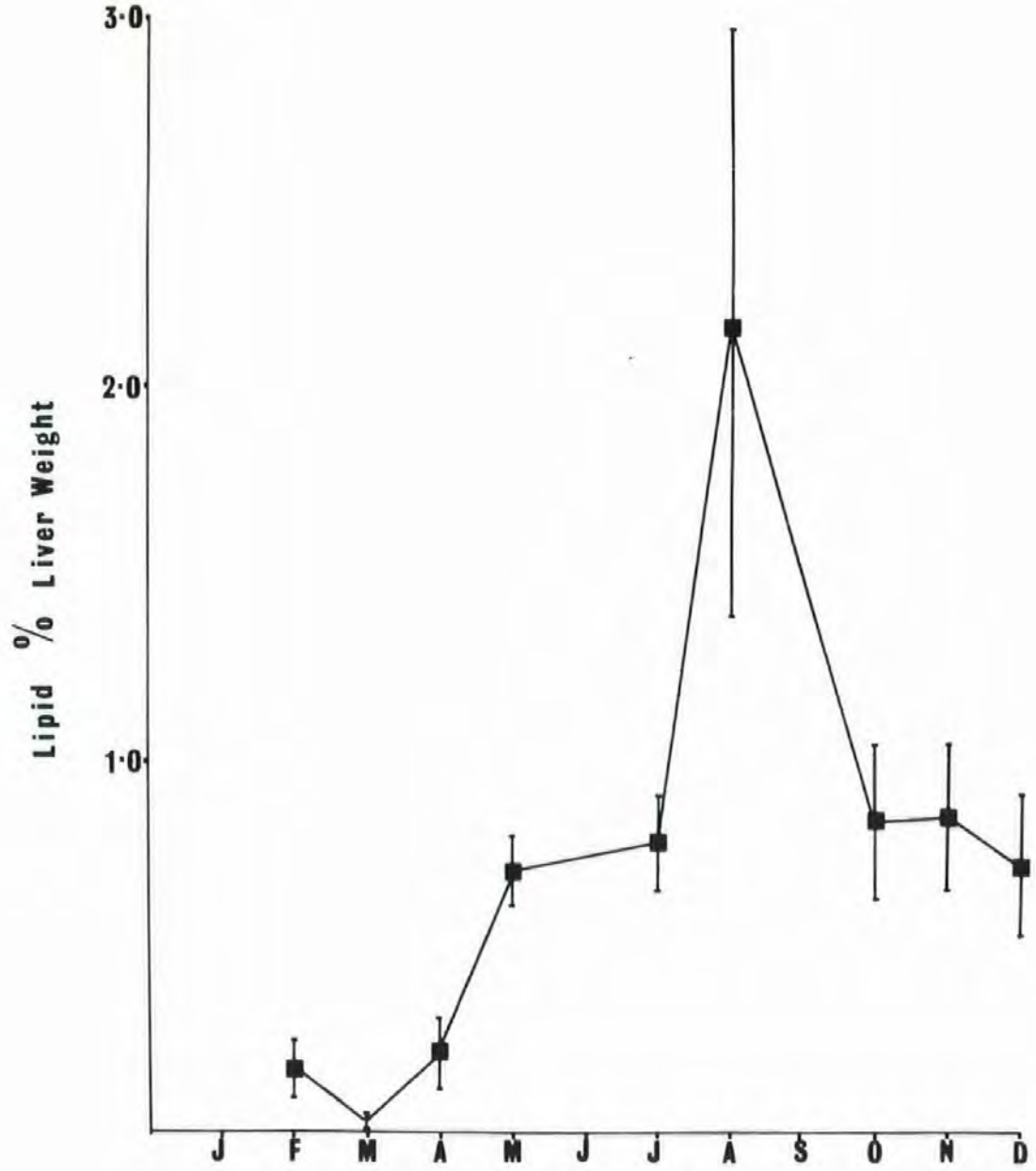

3·0
2·0
1·0
Lipid % Liver Weight
J
F
M
A
M
J
J
A
S
O
N
D

Figure 74 Monthly variation of mean gonad percentage body weight in T.bubalis collected from Portwrinkle.

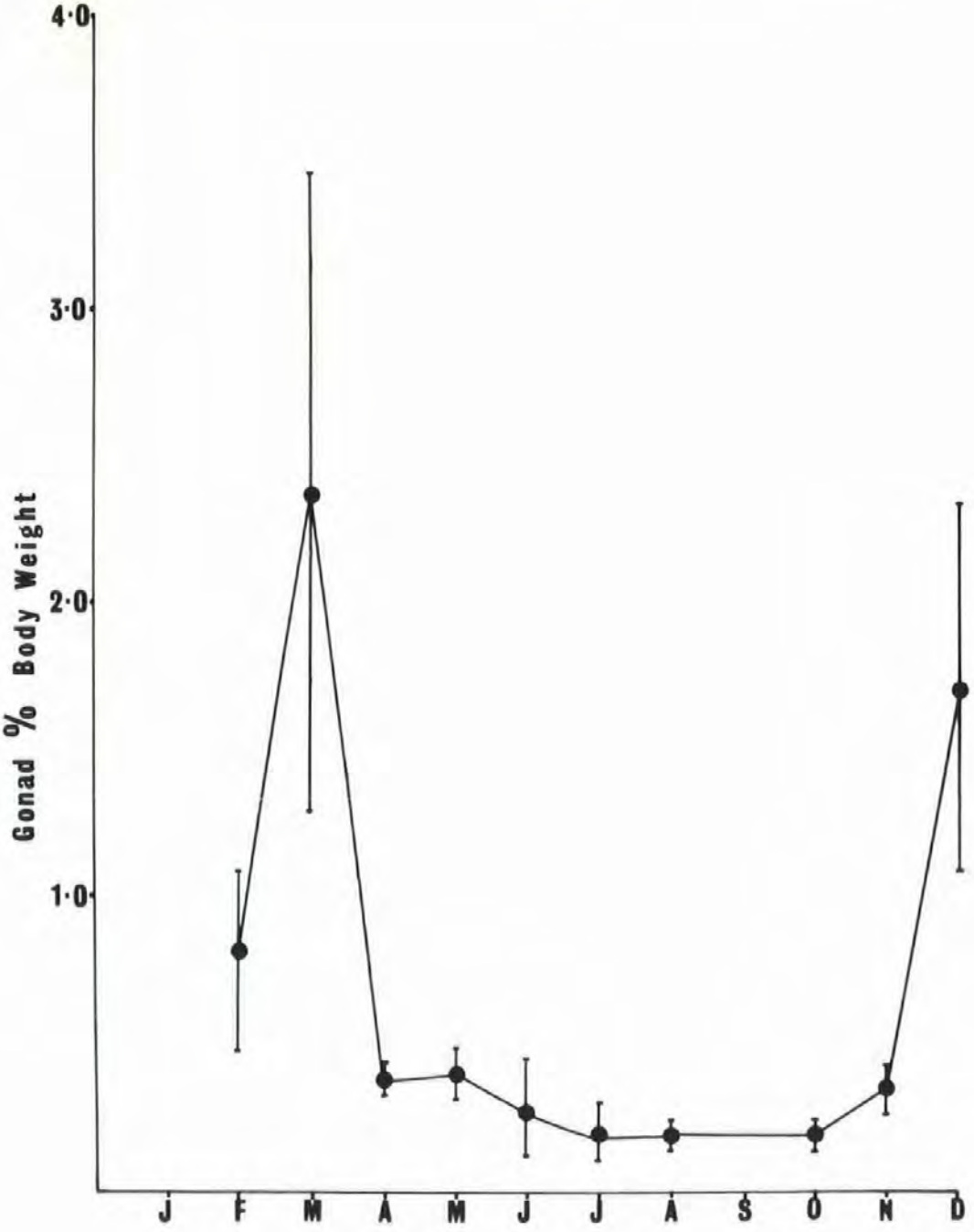

4·0
3·0
2·0
1·0
Gonad % Body Weight
J
F
M
A
M
J
J
A
S
O
N
D

Figure 75a To compare mean host variables of T.bubalis from five sites. (Ab. = Aberystwyth, Wd. = Widemouth, Pt. = Portwrinkle, Wb. = Wembury, R. = Roscoff.

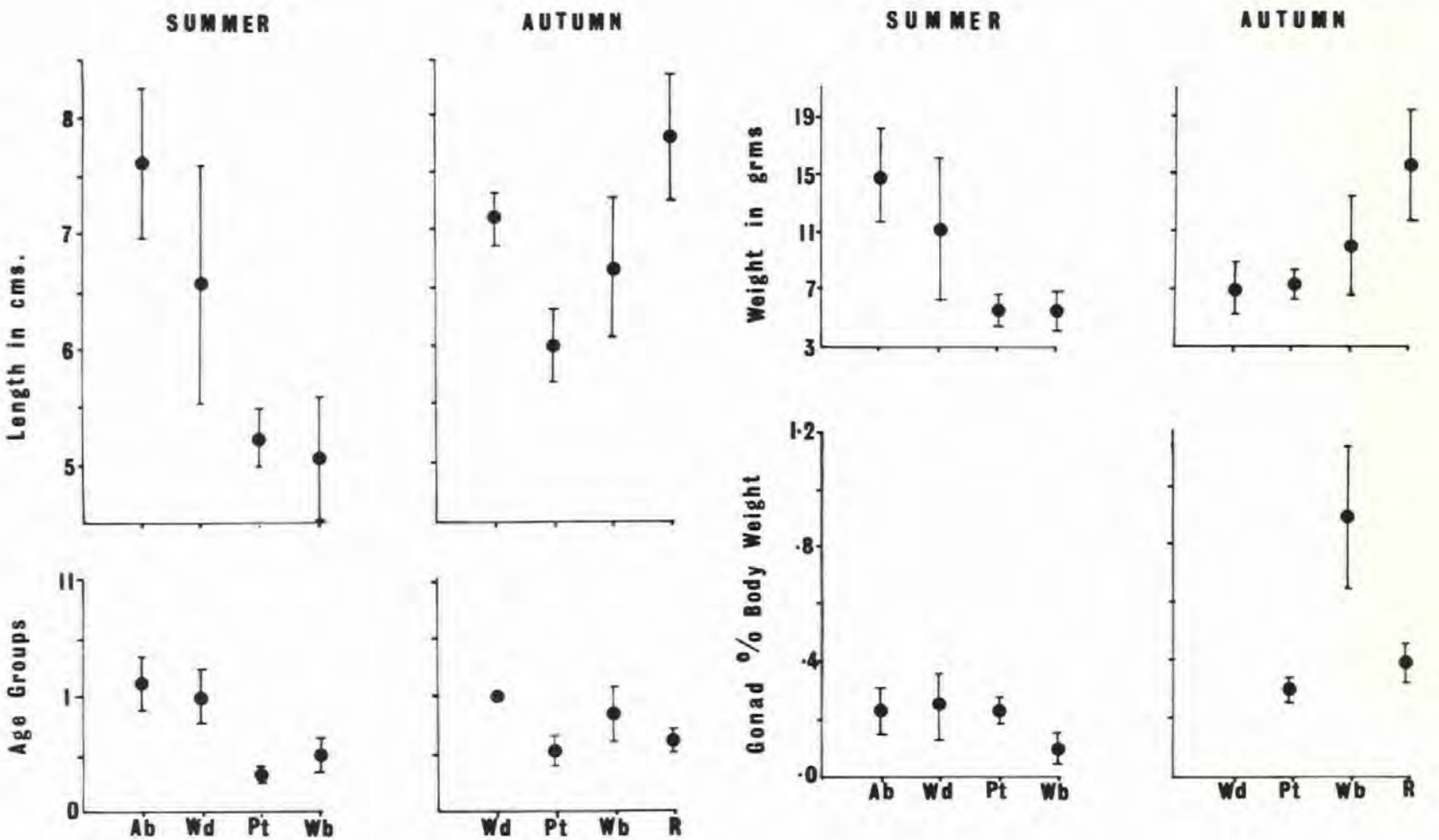

SUMMER
AUTUMN
SUMMER
AUTUMN
Length in cms.
8
7
6
5
Weight in grms
19
15
11
7
3
Age Groups
II
I
0
Gonad % Body Weight
1·2
·8
·4
·0
Ab
Wd
Pt
Wb
Wd
Pt
Wb
R
Ab
Wd
Pt
Wb
Wd
Pt
Wb
R

Figure 75b To compare mean host variables of T.bubalis from five sites. (Ab. = Aberystwyth, Wd. = Widemouth, Pt. = Portwrinkle, Wb. = Wembury, R. = Roscoff)

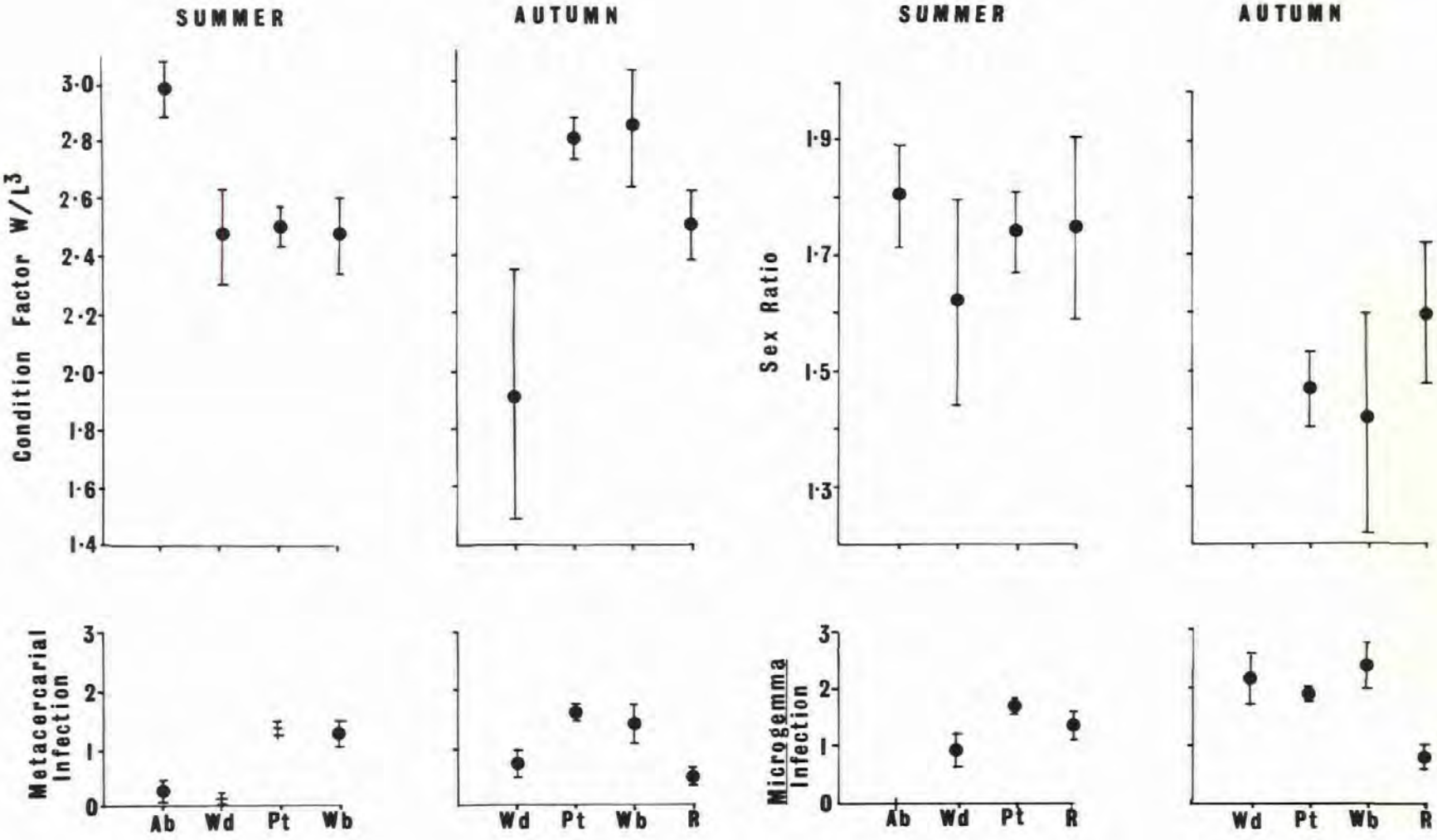

SUMMER
AUTUMN
SUMMER
AUTUMN
Condition Factor W/L3
3·0
2·8
2·6
2·4
2·2
2·0
1·8
1·6
1·4
Sex Ratio
1·9
1·7
1·5
1·3
Metacercarial Infection
3
2
1
0
Ab
Wd
Pt
Wb
Wd
Pt
Wb
R
Microgemma Infection
3
2
1
0
Ab
Wd
Pt
R
Wd
Pt
Wb
R

Figure 75c To compare mean host variables of T.bubalis from five sites. (Ab. = Aberystwyth, Wd. = Widemouth, Pt. = Portwrinkle, Wb. = Wembury, R. = Roscoff)

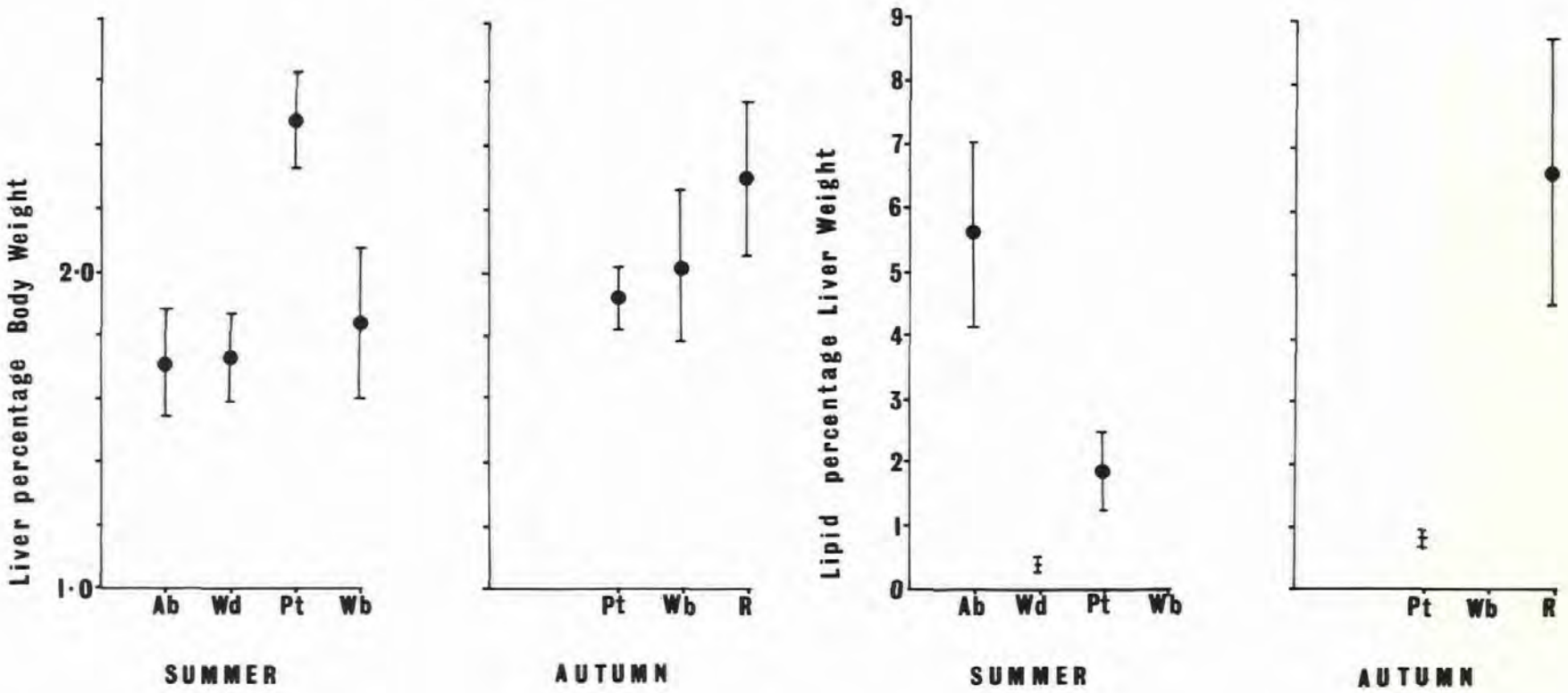

Liver percentage Body Weight
2·0
1·0
Ab
Wd
Pt
Wb
SUMMER
Pt
Wb
R
AUTUMN
Lipid percentage Liver Weight
9
8
7
6
5
4
3
2
1
0
Ab
Wd
Pt
Wb
SUMMER
Pt
Wb
R
AUTUMN

Figure 76 Prevalence of Microgemma infection in the T.bubalis population at Portwrinkle.

Figure 77 Prevalence of hemiurid metacercariae infection in the T.bubalis population at Portwrinkle.

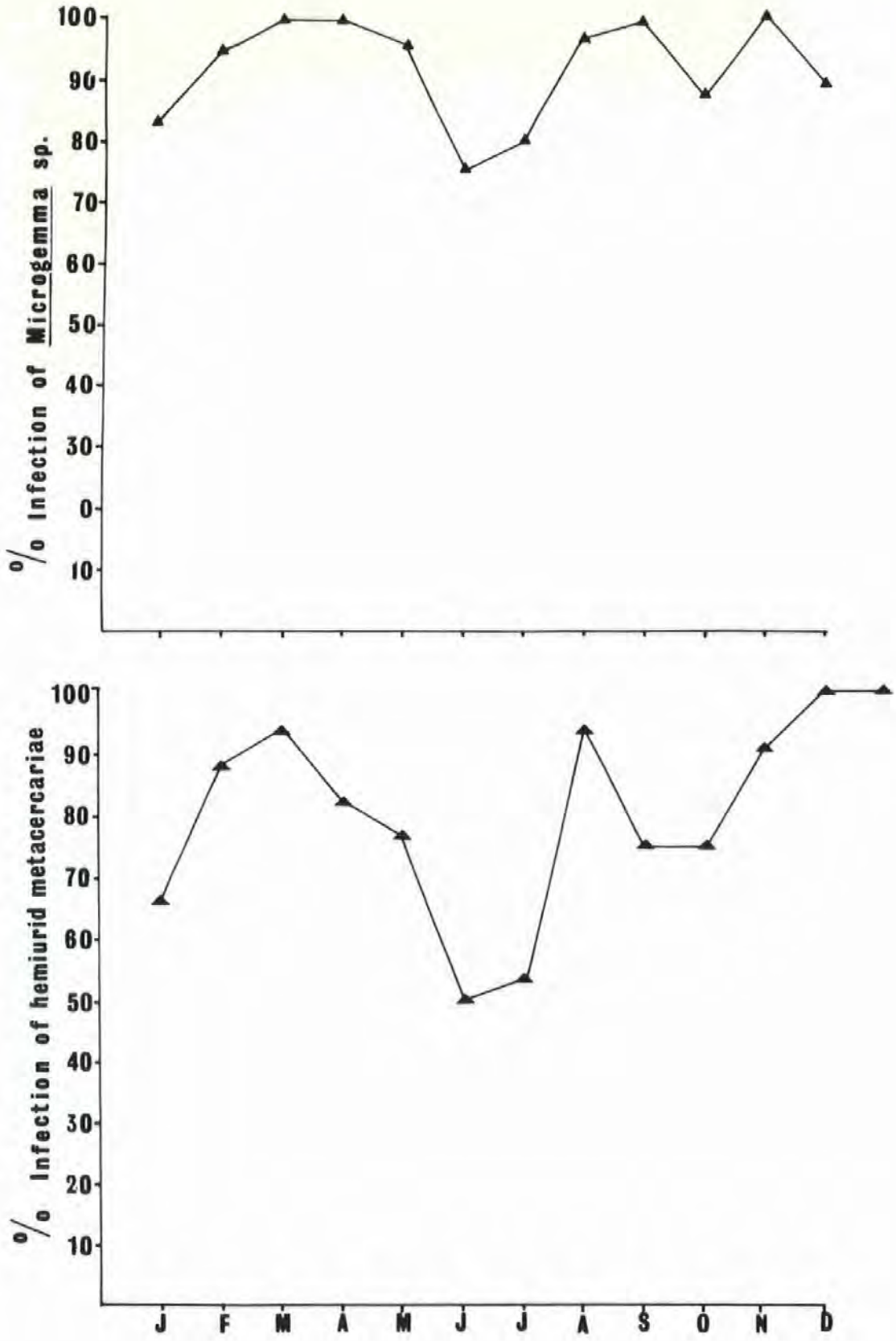

% Infection of Microgemma sp.
100
90
80
70
60
50
40
30
0
10
% Infection of hemiurid metacercariae
100
90
80
70
60
50
40
30
20
10
J
F
M
A
M
J
J
A
S
O
N
D

Figure 78 Monthly variation in the distribution of *Microgemma* infection levels in the *T.bubalis* population at Portwrinkle.

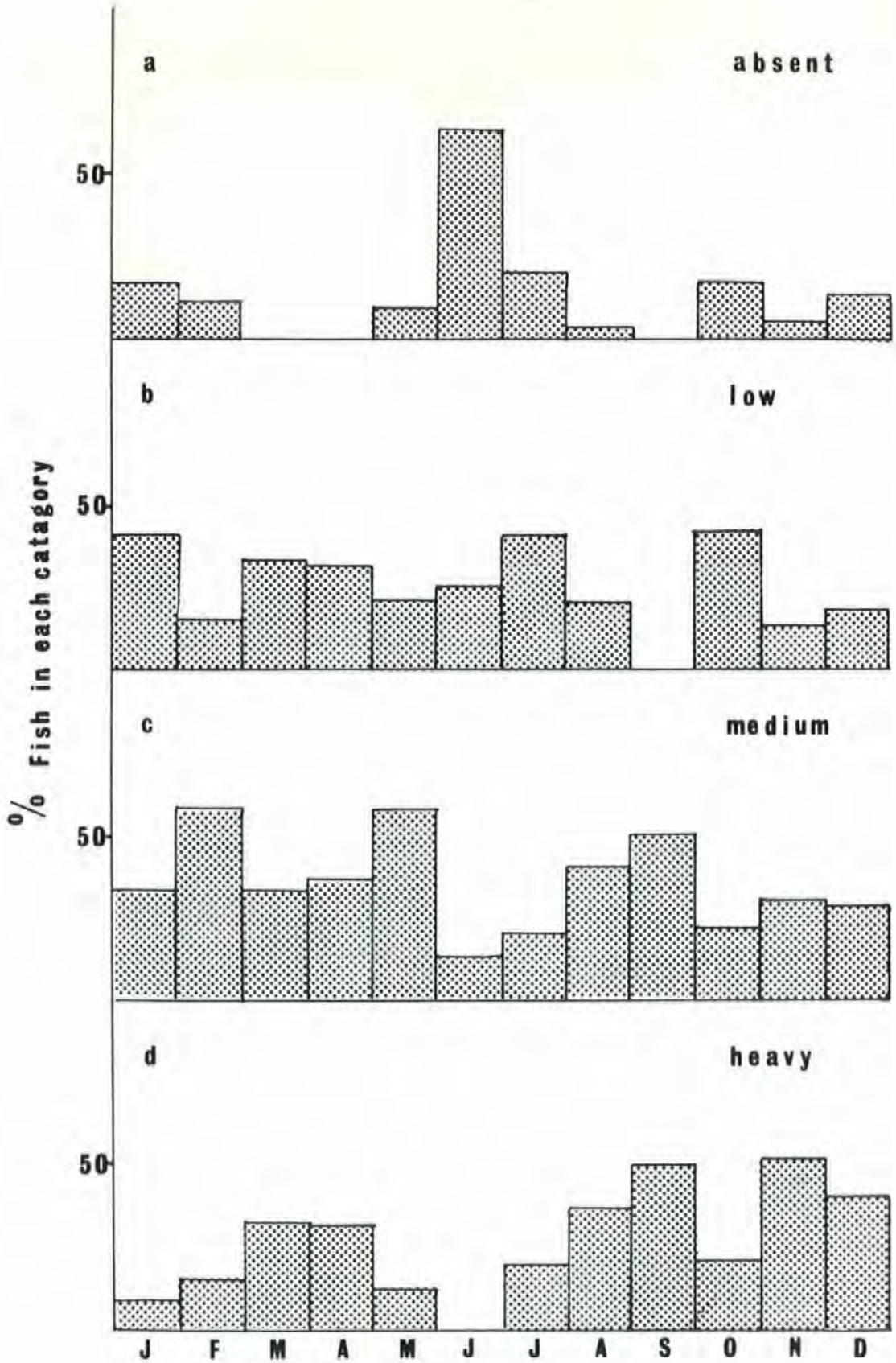

a
absent
50
b
low
50
% Fish in each catagory
c
medium
50
d
heavy
50
J
F
M
A
M
J
J
A
S
O
N
D

Figure 79 Monthly variation in the distribution of hemiurid metacercariae infection levels in the T.bubalis population at Portwrinkle.

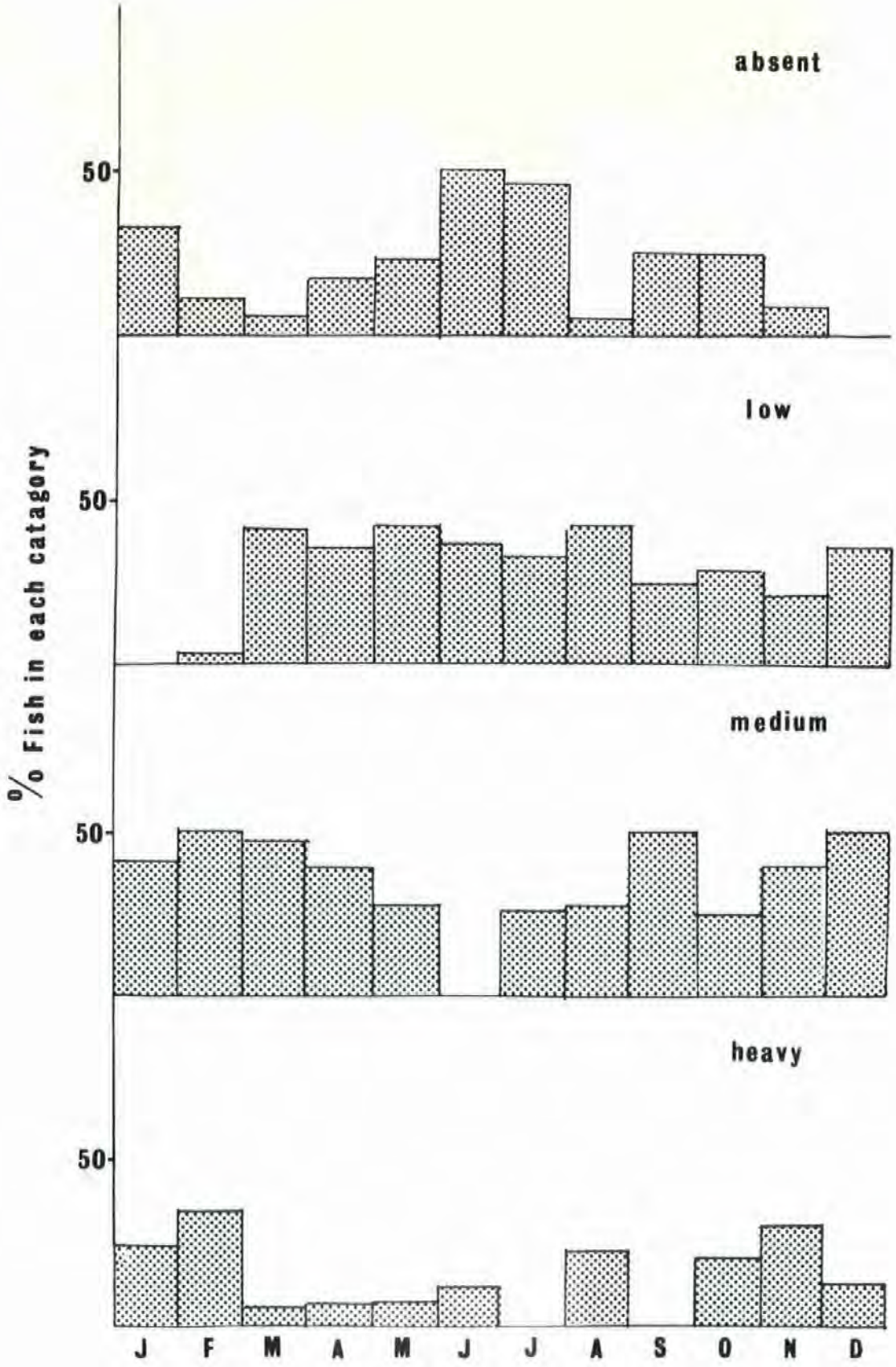

absent
50
low
50
medium
50
heavy
50
% Fish in each catagory
J
F
M
A
M
J
J
A
S
O
N
D

Figure 80 Combined mean monthly infection levels of T.bubalis from Portwrinkle with Microgemma sp. and their relationship with sea temperature.

Figure 81 Combined mean monthly infection levels of T.bubalis from Portwrinkle with hemiurid metacercariae and their relationship with sea temperature.

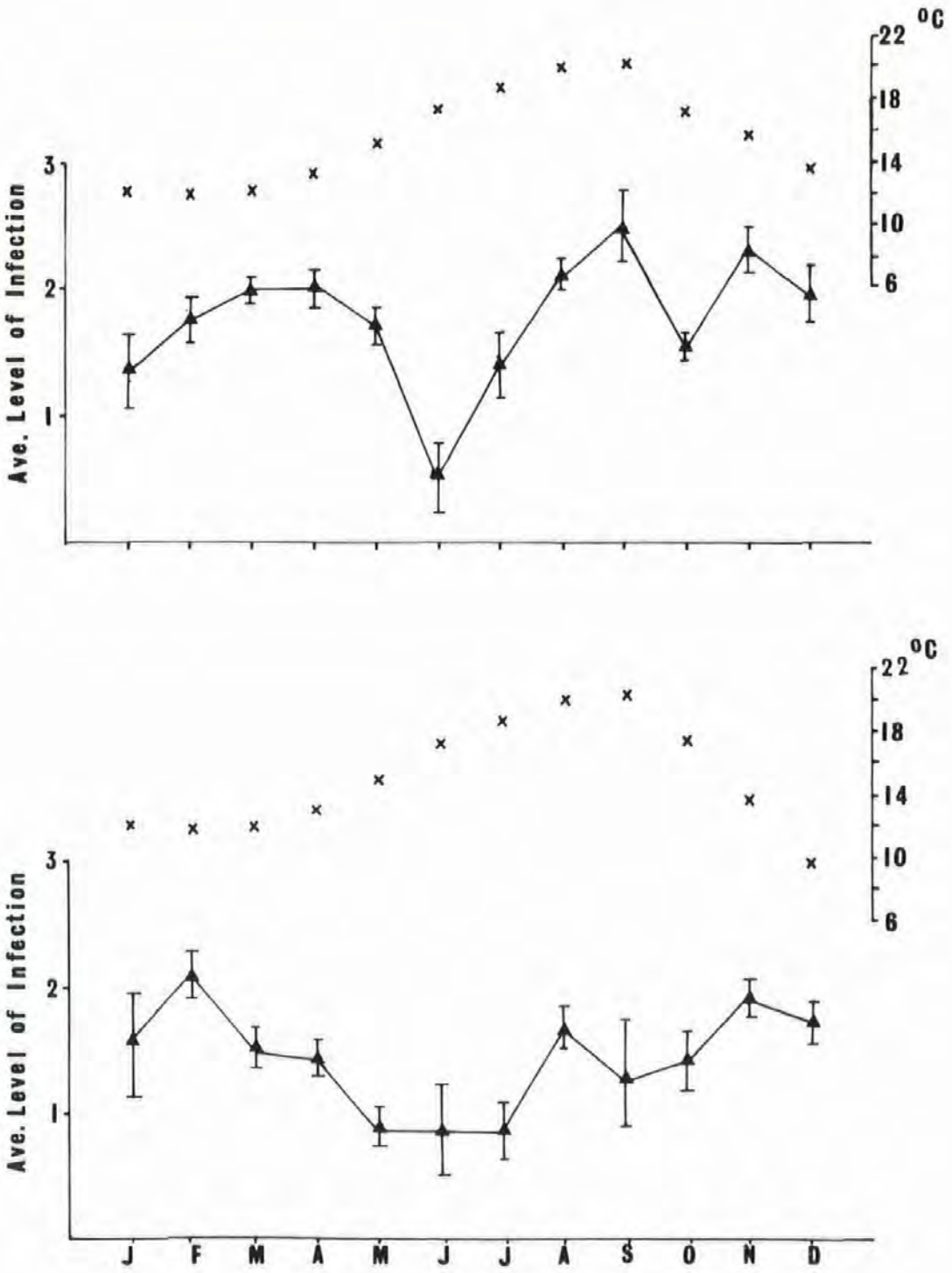

Ave. Level of Infection
3
2
1
22 °C
18
14
10
6
J
F
M
A
M
J
J
A
S
O
N
D

Figure 82 Mean monthly infection levels of T.bubalis from Portwrinkle with Microgemma sp. during the period of study.

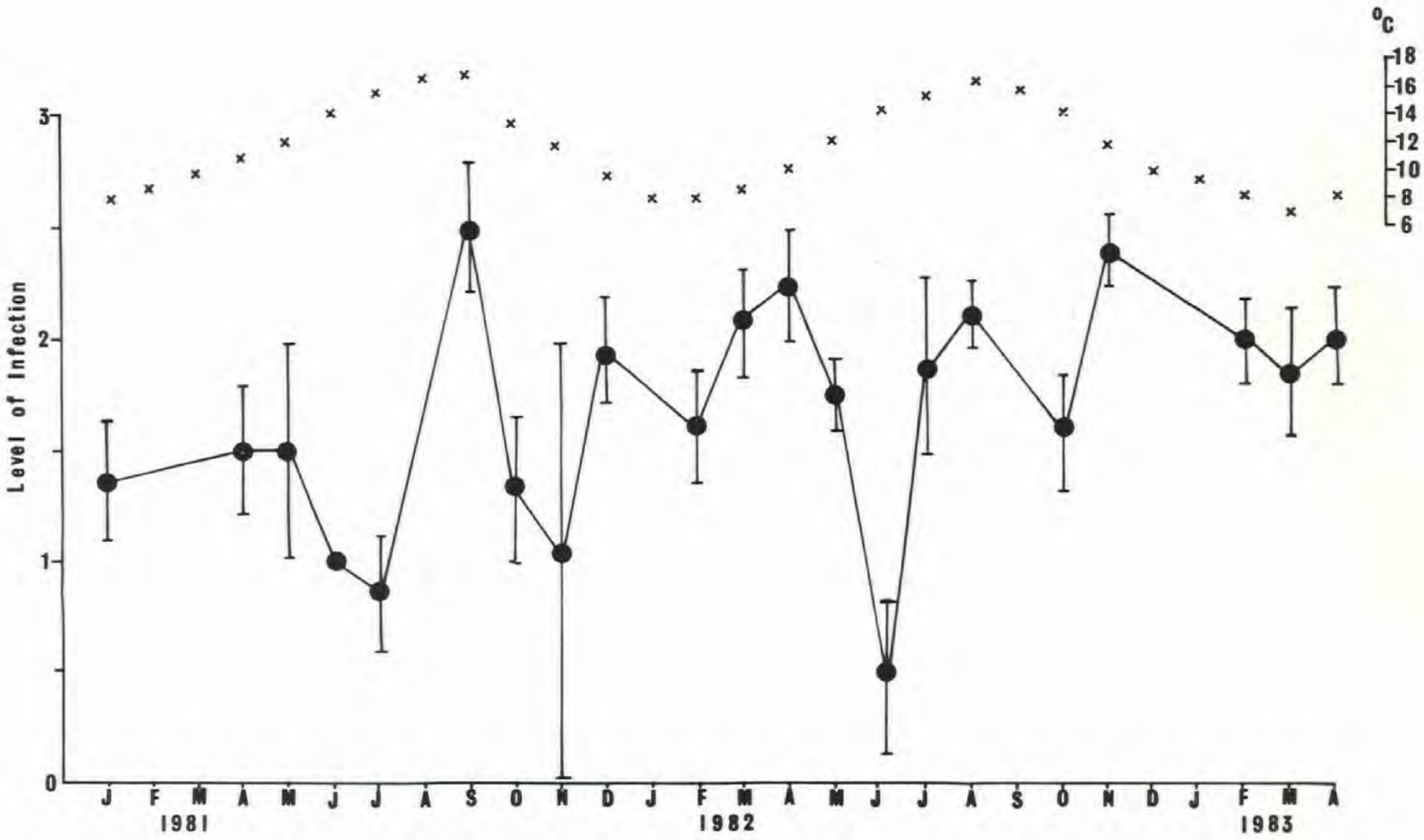

°C
18
16
14
12
10
8
6
Level of Infection
3
2
1
0
J F M A M J J A S O N D J F M A M J J A S O N D J F M A
1981
1982
1983

Figure 83 Mean monthly infection levels of T.bubalis from Portwrinkle with hemiurid metacercariae during the period of study.

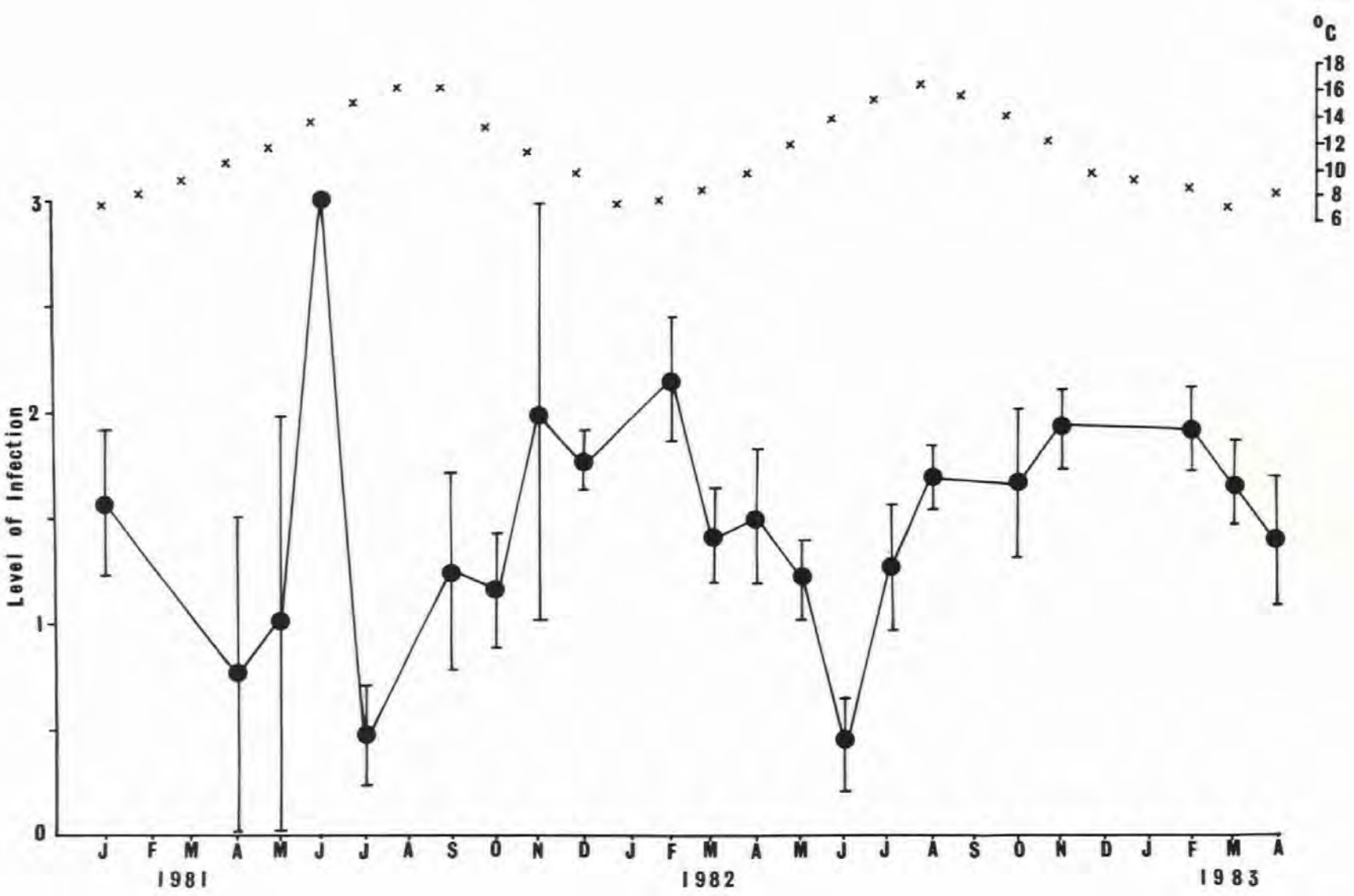

°C
18
16
14
12
10
8
6
Level of Infection
3
2
1
0
J F M A M J J A S O N D J F M A M J J A S O N D J F M A
1981
1982
1983

Figure 84 Variation of mean monthly infection level of *Microgemma* sp. with age of *T.bubalis* from the Portwrinkle sample.

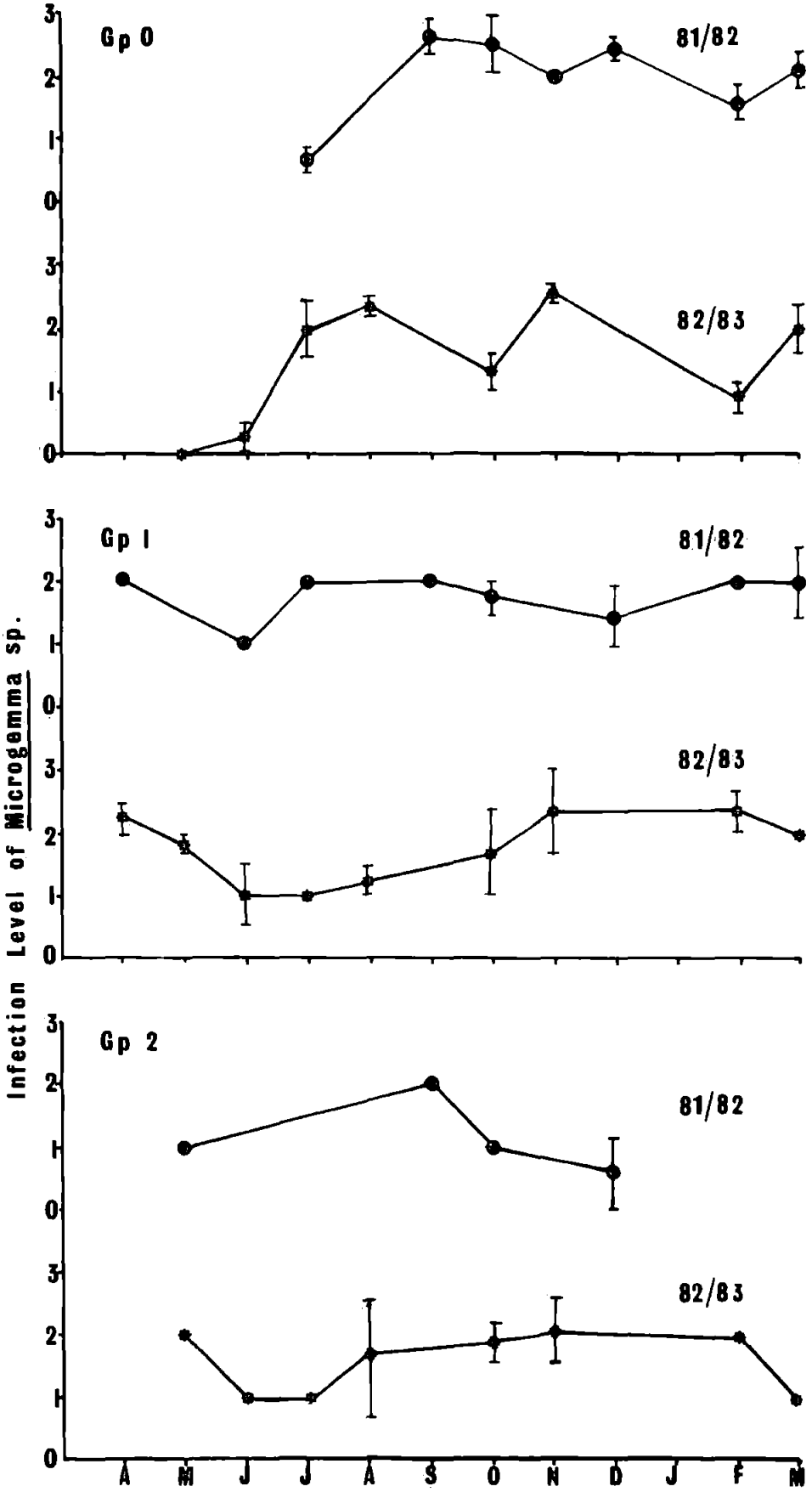

Gp 0
81/82
82/83
Gp 1
81/82
82/83
Gp 2
81/82
82/83
Infection Level of Microgemma sp.
0
1
2
3
A
M
J
J
A
S
O
N
D
J
F
M

Figure 85 Variation of mean monthly hemiurid metacercariae infection with age of T.bubalis from the Portwrinkle sample.

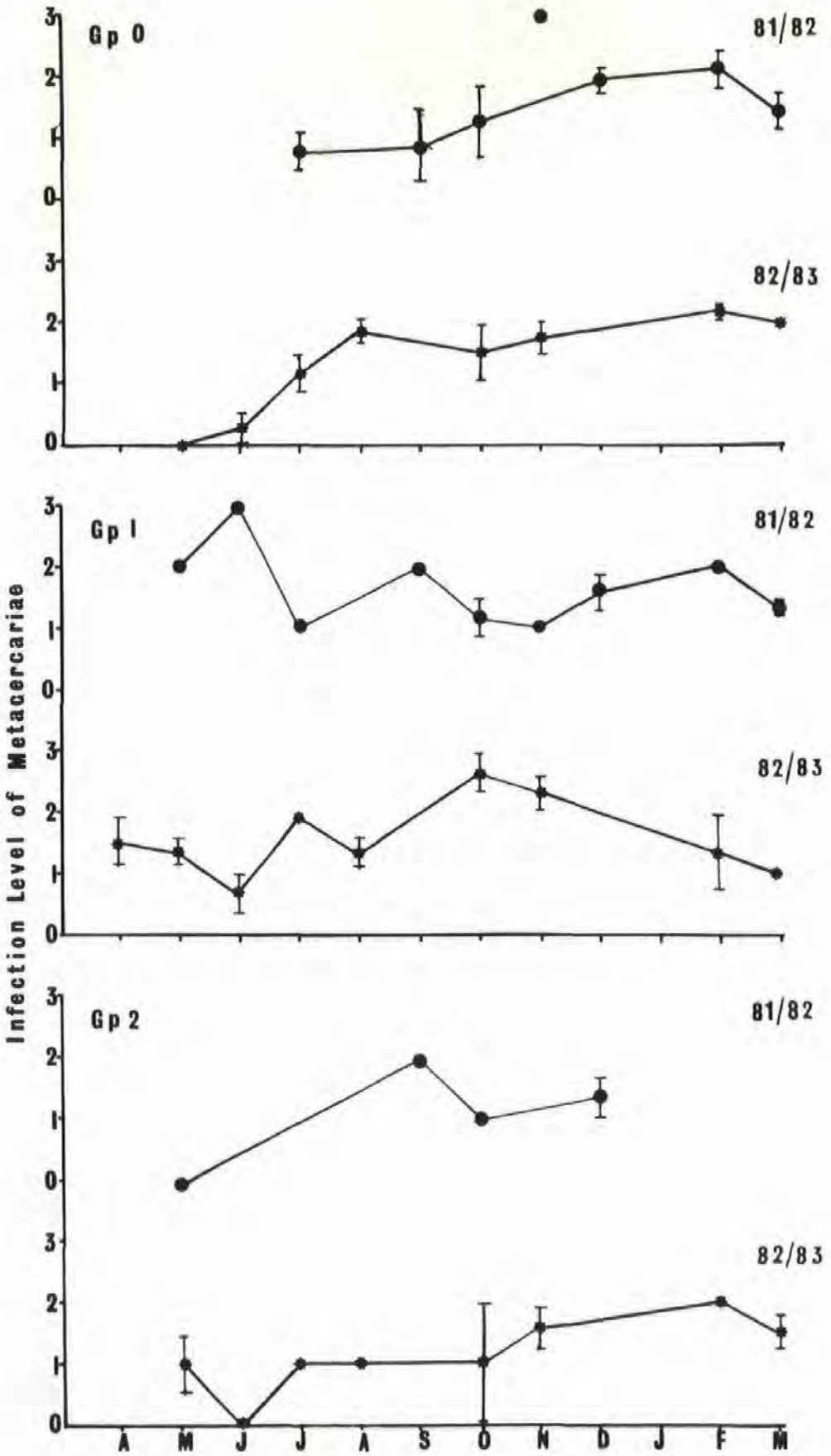

Gp 0
81/82
82/83
Gp 1
81/82
82/83
Gp 2
81/82
82/83
Infection Level of Metacercariae
0
1
2
3
A
M
J
J
A
S
O
N
D
J
F
M

Figure 86 Variation in mean monthly infection levels of *Microgemma* sp. for each generation of *T.bubalis* sampled from the Portwrinkle population.

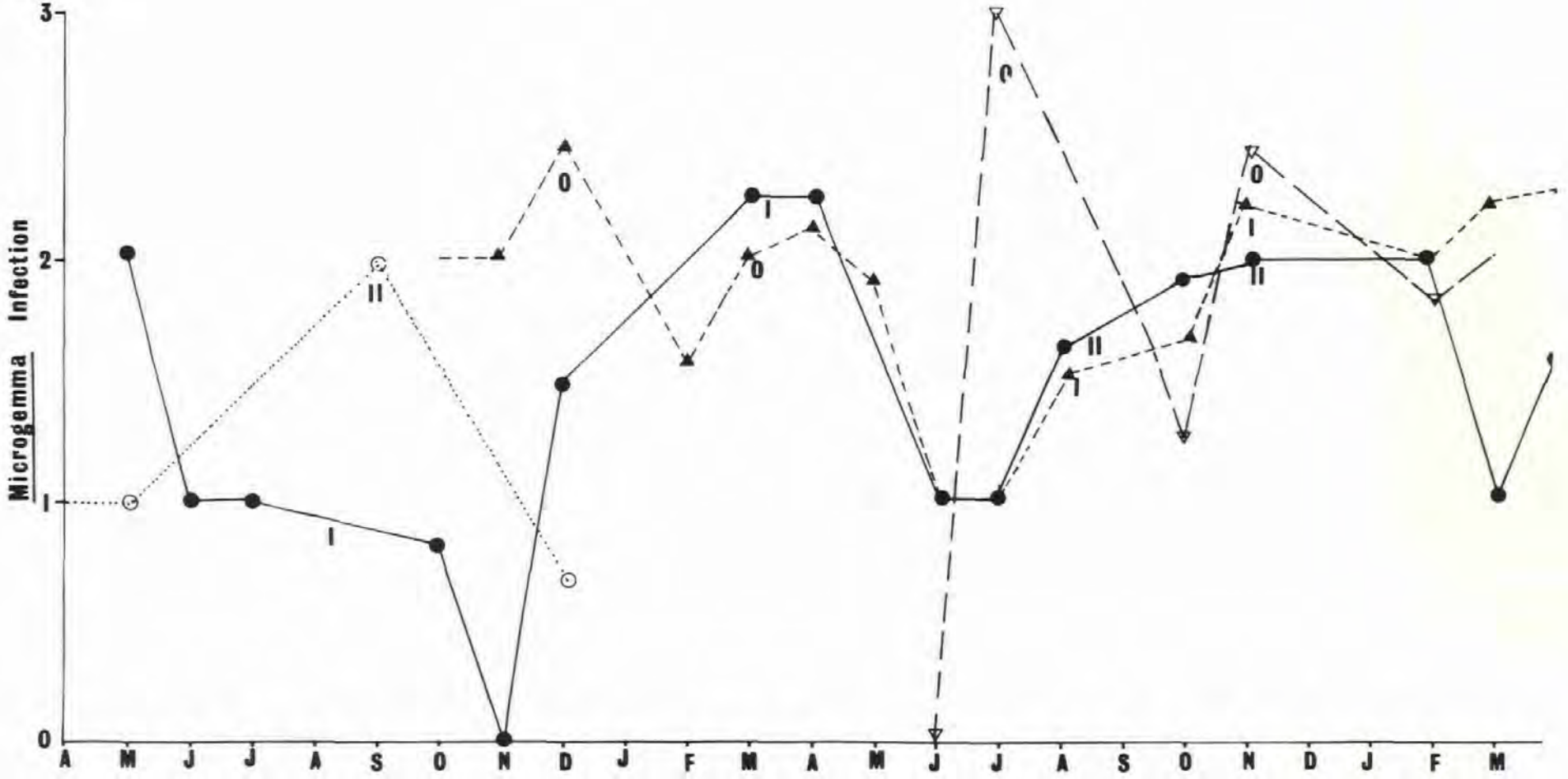

Microgamma Infection
3
2
1
0
A M J J A S O N D J F M A M J J A S O N D J F M
0
I
II

Figure 87 Variation in mean monthly infection levels of hemiurid metacercariae for each generation of T.bubalis sampled from the population at Portwrinkle.

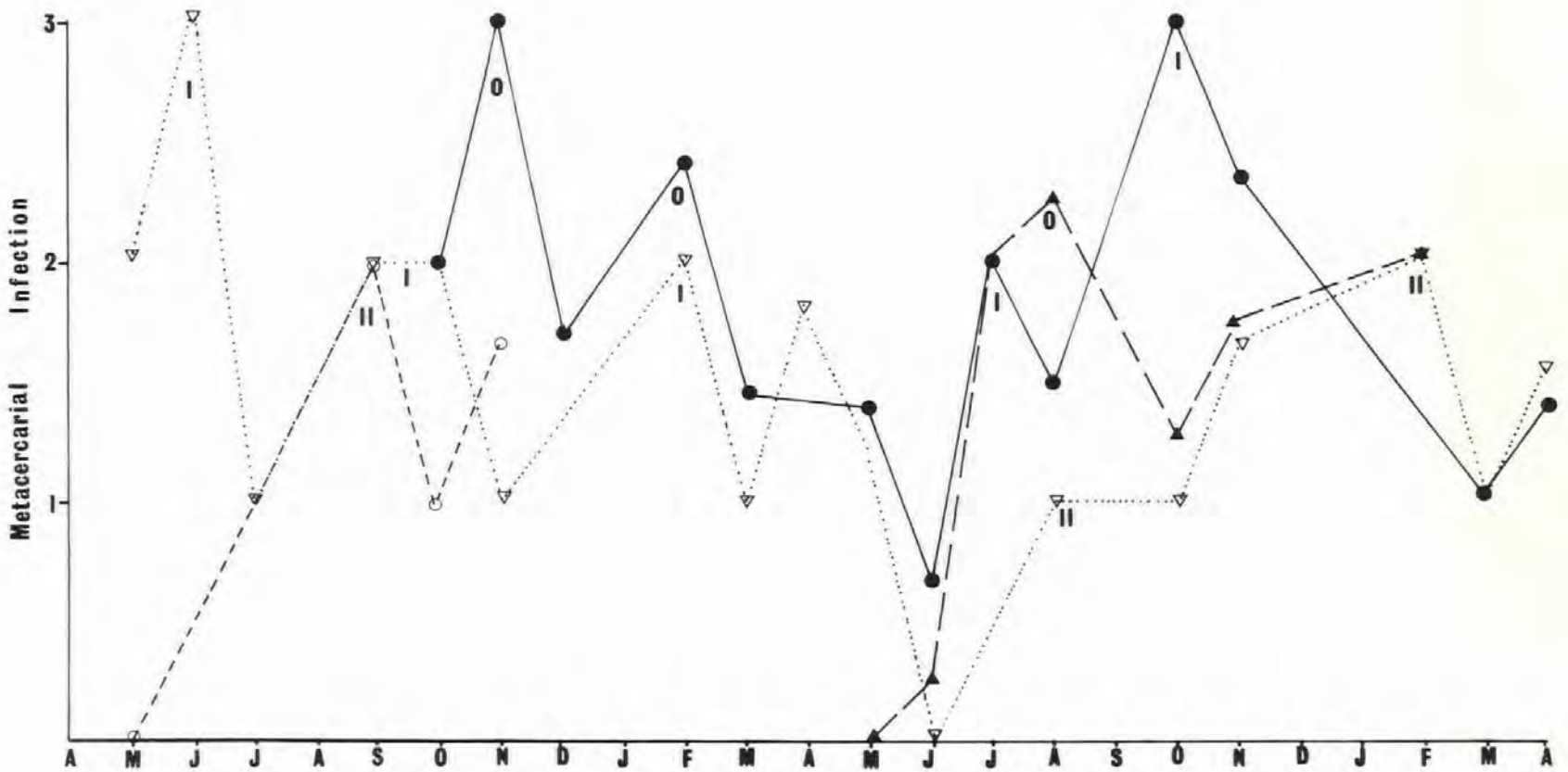

Metacercarial Infection
3
2
1
A M J J A S O N D J F M A M J J A S O N D J F M A
I
II
I
II
I
II
I
II
I
II
I
II
I
II
I
II
I
II
I
II
I
II
I
II
I
II
I
II

Figure 88 Variation of mean monthly infection levels with Microgemma sp. for the male and female population of T.bubalis collected from Portwrinkle.

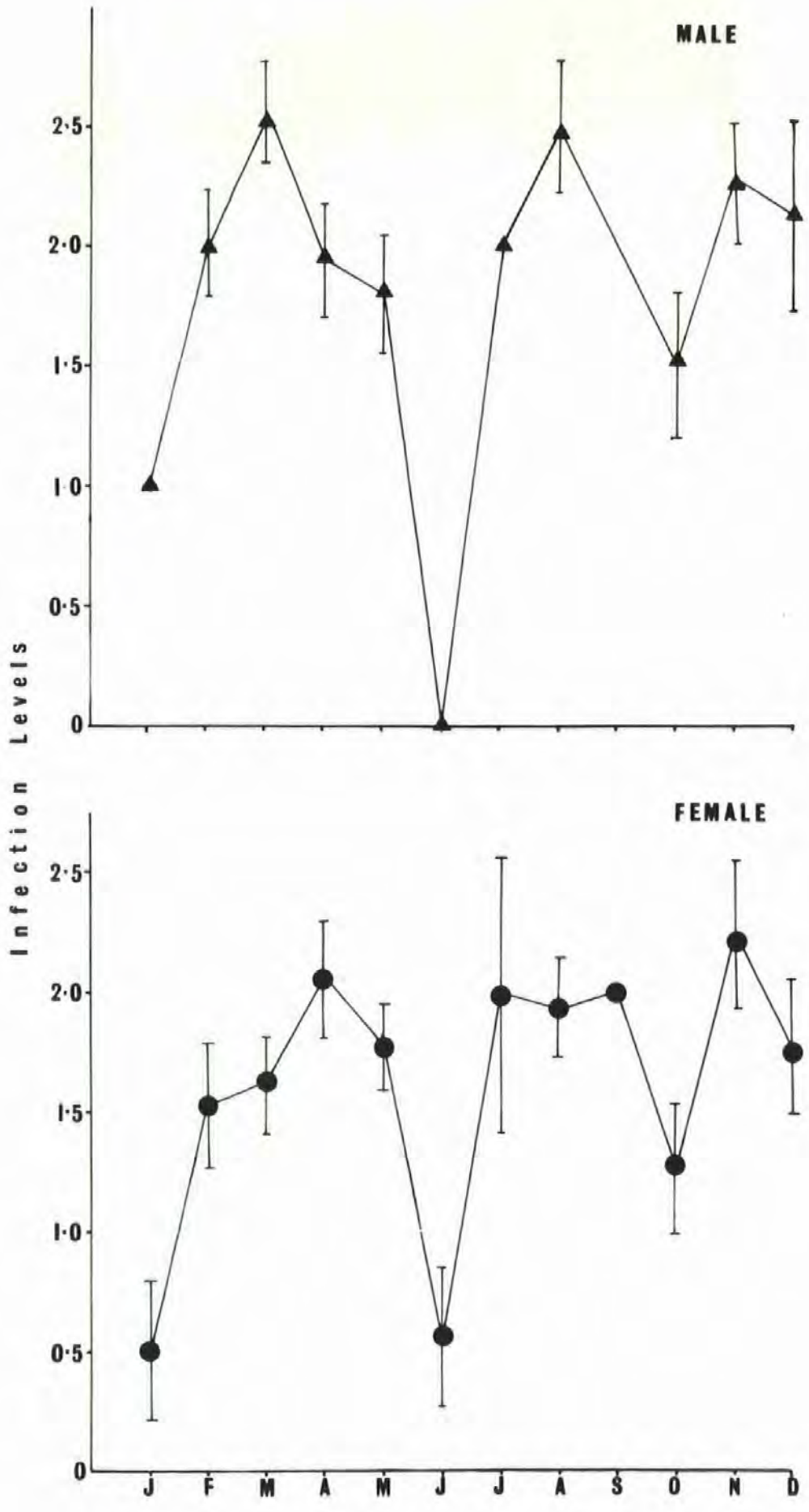

MALE
FEMALE
Infection Levels
2·5
2·0
1·5
1·0
0·5
0
J
F
M
A
M
J
J
A
S
O
N
D

Figure 89 Variation of mean monthly infection levels with hemiurid metacercariae for the male and female population of T.bubalis collected from Portwrinkle.

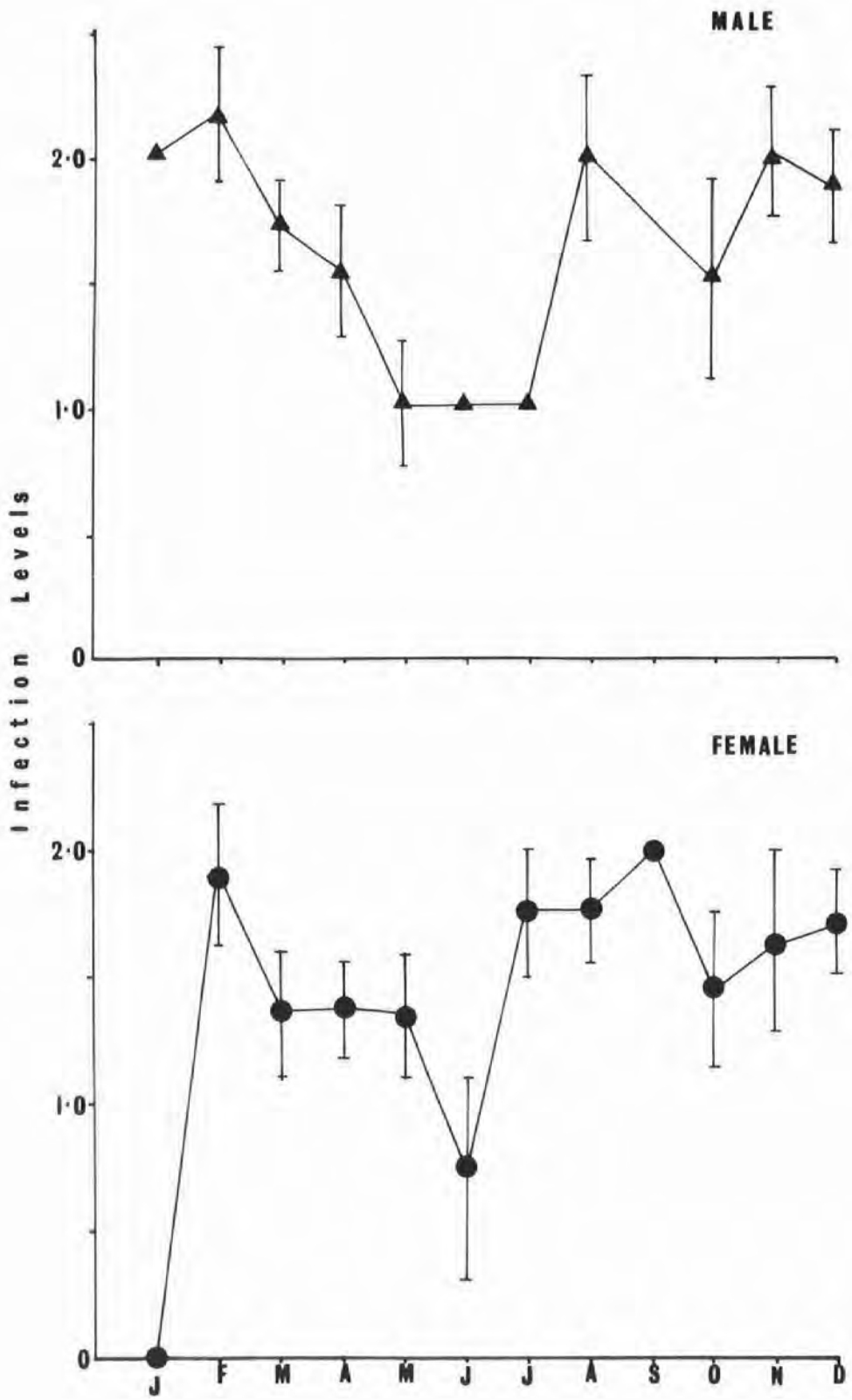

MALE
FEMALE
Infection Levels
2·0
1·0
0
J
F
M
A
M
J
J
A
S
O
N
D

Figure 90 To show the proportion of T.bubalis from the Portwrinkle sample infected with a) Microgemma sp. and b) hemiurid metacercariae for each age/sex combination.

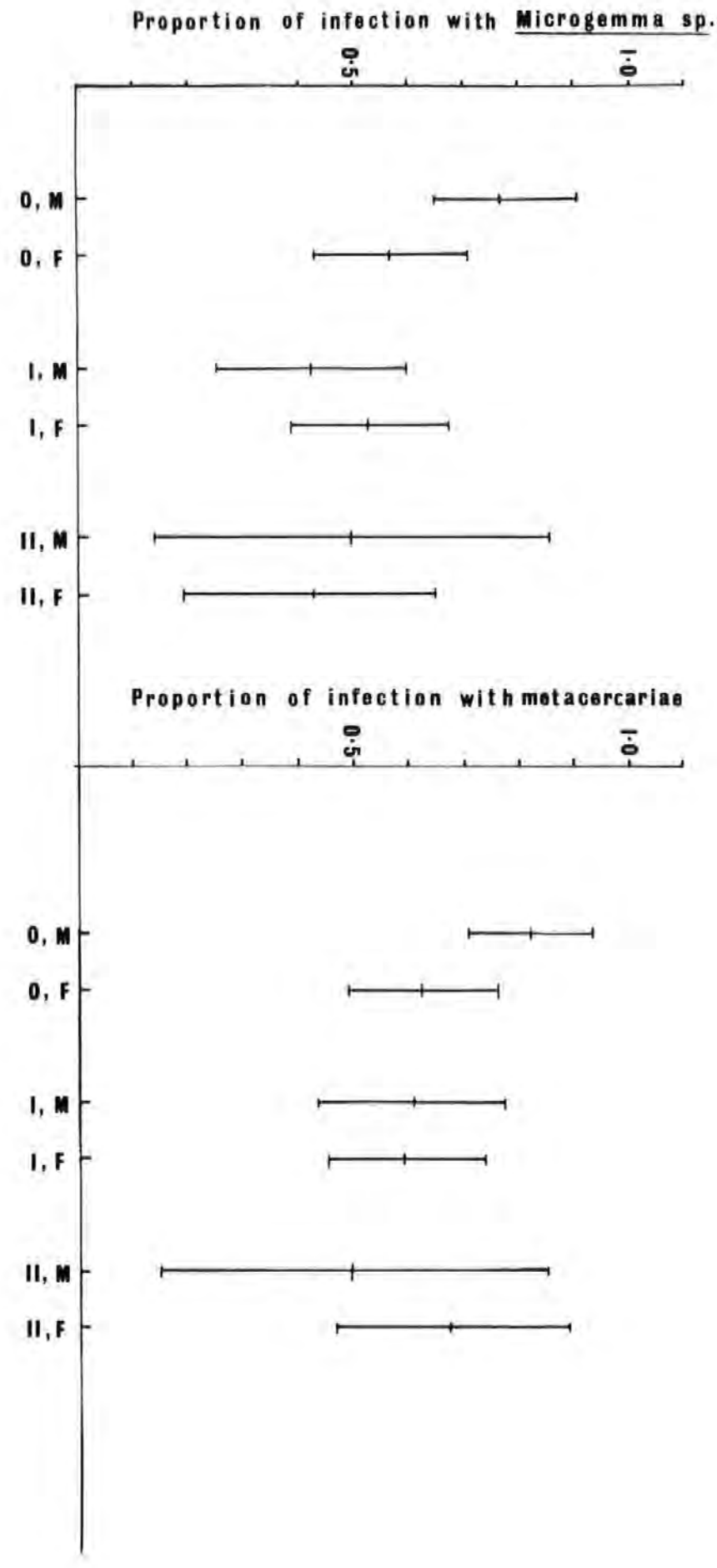

Proportion of infection with Microgemma sp.
0·5
1·0
0, M
0, F
I, M
I, F
II, M
II, F
Proportion of infection with metacercariae
0·5
1·0
0, M
0, F
I, M
I, F
II, M
II, F

Figure 91 To illustrate positive interactions between heavy and light infections of Microgemma sp. and host variables of the T.bubalis population collected at Portwrinkle. (Shaded area = observed percentage, unshaded area = expected percented).

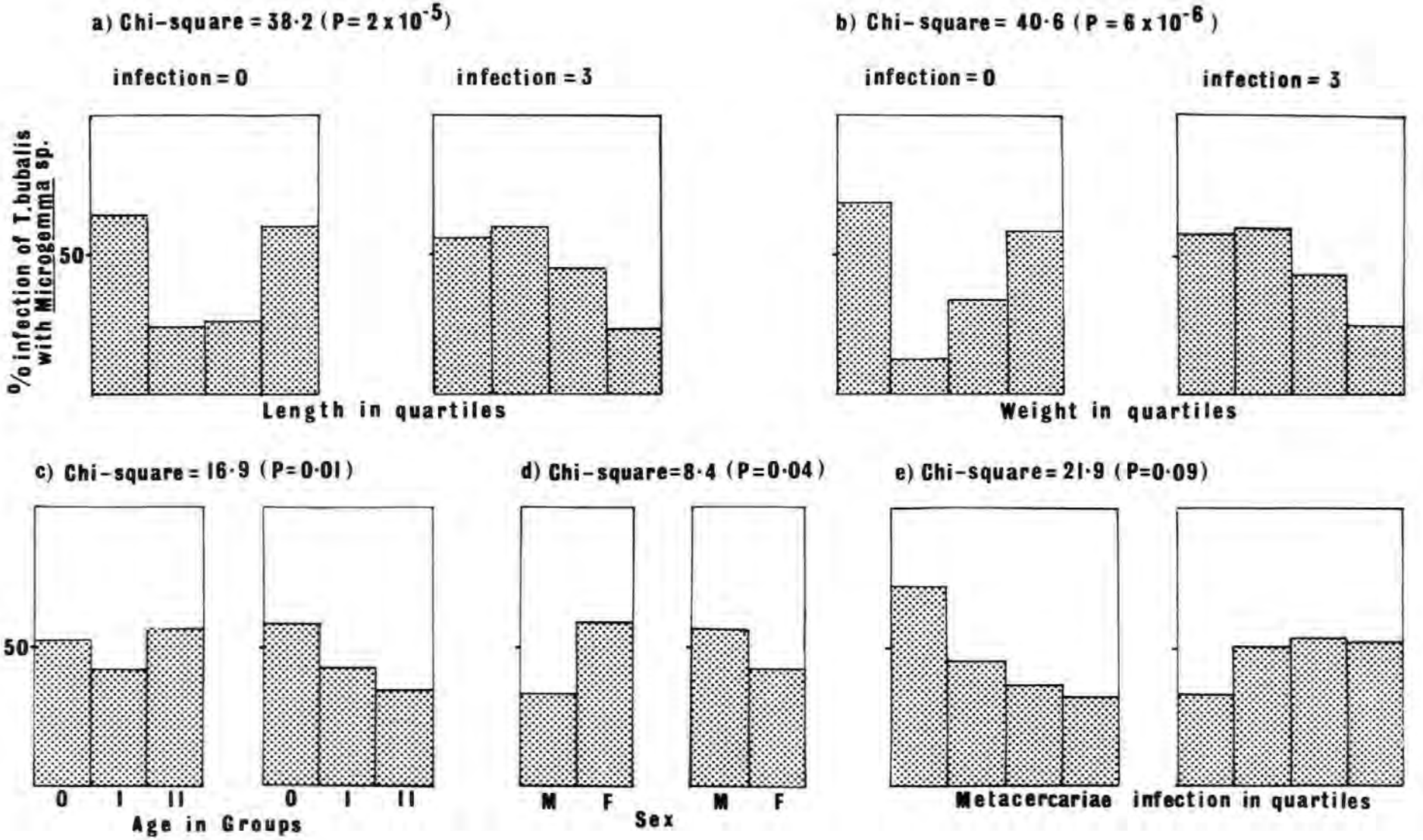
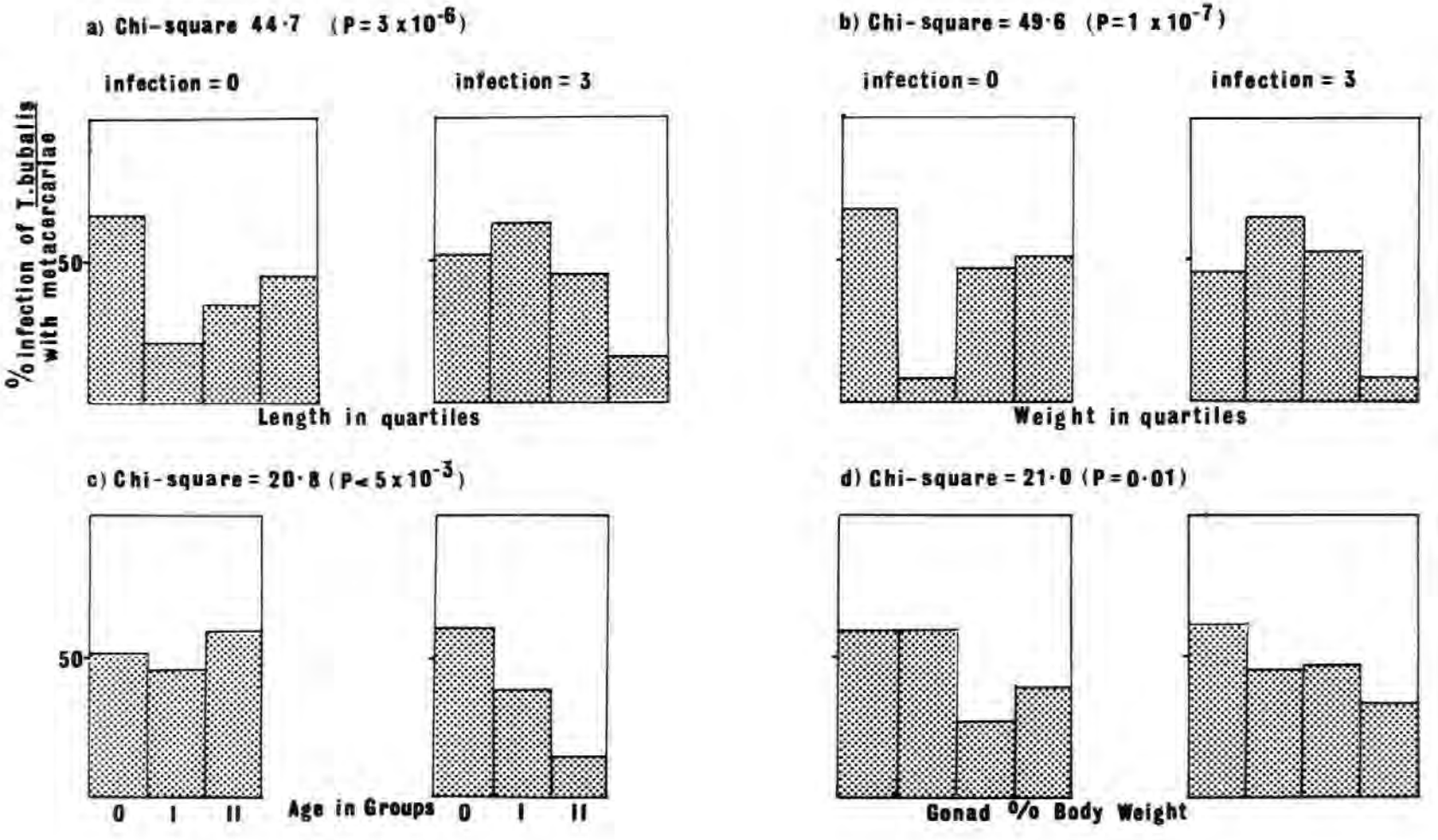

a) Chi-square = 38·2 (P= 2 x 10-5)
infection = 0
infection = 3
% infection of T.bubalis with Microgemma sp.
50
Length in quartiles
b) Chi-square = 40·6 (P = 6 x 10-6)
infection = 0
infection = 3
Weight in quartiles
c) Chi-square = 16·9 (P=0·01)
50
0 I II 0 I II
Age in Groups
d) Chi-square=8·4 (P=0·04)
M F M F
Sex
e) Chi-square = 21·9 (P=0·09)
Metacercariae infection in quartiles

Figure 92 To illustrate positive interactions between heavy and light infections of hemiurid metacercariae and host variables of the T.bubalis population collected at Portwrinkle. (Shaded area = observed percentage, unshaded area = expected percentage).


a) Chi-square 44·7 (P = 3 x 10⁻⁶)
infection = 0
infection = 3
% infection of T.bubalis with metacercariae
50
Length in quartiles
b) Chi-square = 49·6 (P = 1 x 10⁻⁷)
infection = 0
infection = 3
Weight in quartiles
c) Chi-square = 20·8 (P < 5 x 10⁻³)
50
0 I II
Age in Groups 0 I II
d) Chi-square = 21·0 (P = 0·01)
Gonad % Body Weight

D I S C U S S I O N

Microgemma sp. appears to be confined chiefly to the southerly range of host species Taurulus bubalis, the highest infection here being recorded in south west Britain and Brittany with lower levels to the north at Aberystwyth. The literature survey supported this view there being no records of hepatic microsporidia in this host or indeed in any other species of teleost north of the present investigation (Sprague 1977). It seems unlikely that the levels of the intensity recorded here would have gone unnoticed as the parasitology of marine fishes in norther European waters has received a relatively high degree of attention.

HOST

A knowledge of host ecology is a prerequisite in attempting an epidemiological investigation of any parasitic disease. It is relevant here therefore to discuss aspects of the biology of Taurulus bubalis. A population from Portwrinkle was selected here for a detailed investigation as it is assumed to be a representative community. Lamp (1966) undertook a similar study on a population from the Kiel Fjord which, despite some differences, provided a valuable comparison. It was necessary however before analysing this difference to evaluate the collecting techniques which in the case of Lamp (1966) failed to sample the juvenile population. To some extent the habitat dictated the method used. In the present study catches were only made in rockpools enabling the anaesthetic quinaldine to be used for collecting purposes. Using this method it

was possible to sample the whole population of the pool. In contrast the population sampled from the open water of the Kiel Fjord necessitated the use of traps. Although this was successful in catching large samples the failure to collect fish less than 7.5cm in length excluded the '0' group fish from the study.

This investigation confirms the view of Lamp (1966) that Taurulus bubalis is a short lived species. No fish older than four years being recorded here. The largest fish recorded from the survey measuring 16.8cm belonged to this age group and was only 0.7cm short of the record for the species (Wheeler 1969). 81% of the Portwrinkle population were less than three years, group '0' and 'I' making up 43% and 38% respectively. The abrupt decline in population by age groups II + was also recorded by Lamp (1966) and in agreement with him is considered to result from mortality rather than migration.

That the sex ratio is here shown to increase with age in favour of the females agrees with Lamp (1966). A ratio 1 : 1 in the '0' group population increases to 3 : 2 in groups 'I' and 'II'. The resulting decline in the male population could be attributed to mortality resulting from post spawning stress. Adverse weather conditions at this time of year being likely to emphasise this. The relatively small size of the male Taurulus bubalis which seldom exceeds lengths greater than 8cm with a maximum of 12cm here could be associated with earlier gonad development, resources being diverted into spermatogenesis rather than somatic growth in the first year. The breeding season is recognised by the seaward migration of mature fish to the

lower shore to spawn. It is generally accepted that eggs are guarded by the parent, a view that is further supported by internal fertilization (Lamp 1966) with the production of relatively few eggs. Observations by various workers notably Russel (1976), Le Danois (1913) and Thomopoulos and Bauchot (1975) indicate that either sex may carry out this task.

The two distinct peaks of gonad weight observed in Taurulus bubalis at Portwrinkle was also recorded by Lamp (1966) who attributed it to the successive spawning of individual fish. It was not possible to confirm this here as the likelihood of the peaks belonging to successive generations could not be discounted without further experimental evidence. There are approximately equal ratios of mature males to females, the early maturation of the male in its first year ensuring additional gene flow as would secondary spawning. Other factors favouring secondary spawning would be the greater fecundity provided to a short lived species, and the increased chances of egg survival in the event of adverse conditions. The low water springs zone where the eggs are deposited proved the most difficult region to sample and it was not possible to determine the distribution or incubation period of the eggs. According to Ehrenbaum (1904) the eggs hatch after approximately six weeks dependant on environmental conditions such as sea temperature, and then the larvae are carried into the plankton. Detection of spent fish are indicative of spawning between January and March which agrees with observations by Cunningham (1891) in this area. The presence of larvae in the plankton between March and June fitted in well with the estimated embryonation period which is dependant

to some degree on environmental conditions.

Taurulus bubalis is a major predator in the rockpool environment, second only perhaps to the conger eel. Its reputation as a voracious feeder was confirmed from observations in the aquaria, its choice of food being limited only by size with the ability to consume 50% of its own body weight (Western 1968) and not being adverse to cannibalism. The stomach contents of juvenile fish commonly revealed Nebalia bipes and Dynamera bidentata, whereas the bulk of the adult diet was made up of Idotea baltica, small crabs and fish. As a result of a varied diet it might be expected that Taurulus bubalis will be exposed to a wide range of parasitic infections, dependant on crustacean and fish transport and intermediate hosts. The age of the fish will also affect parasitic infection by influencing habitat, post-larval stages being confined to the plankton whilst juvenile fish exploit the mid water region. In contrast however, the adults are essentially adapted to a demersal way of life, their feeble pectoral fins restricting their manoeuvrability in mid water (Western 1968).

Although lipid is considered an essential source of metabolic energy within fish nutrition (Lee and Sinnhuber 1972) there is little information concerning the role of the liver as a storage site for lipid. The relative importance of the liver as a store has been questioned by some workers notably Dawson and Grimm (1980) who recognised that within non fatty fish such as Pleuronectids and Gadoids the principal reserves of fat are stored within the carcase. Nevertheless these fish store an appreciable amount of lipid in the liver, values of up to 8% being recorded here

from I.bubalis. It is known that these reserves are utilized as may be expected in times of stress. Hines & Spira (1974) detecting significant fat losses from the liver of carp suffering from Ichthyophthiriasis. In the present study it was shown that hepatic lipid fluctuated with season and to some extent with gonad growth, a serious depletion of lipid in September therefore being expected to influence fecundity.

Condition and development of the liver was here assessed on the basis of liver percentage body weight, the value of which may fluctuate according to age and condition factor of the fish. The development of body musculature with age might be expected to play an increasing role in the storage of nutrients, results here showing a decrease in liver percentage body weight with age. Condition factor was considered to be an appropriate measure of condition and is likely to be influenced by many factors including disease, starvation and breeding. These factors could well account for the peak values of liver percentage $\frac{\text{body weight}}{\text{occurring}}$ in June when the majority of the sample were '0' group, and in August and November/December when peak levels of metacercarial infection were recorded. It was interesting to note that the peak value of condition factor proceeded that of lipid percentage body weight. This suggests that unlike hepatic lipid which might directly reflect the amount of food consumed, condition factor reflects the result of assimilated food which may also be affected by maturation and disease and environmental stress.

As mentioned previously sampling here was confined to a limited range within the southern mid region of the host

distribution between 48°N.53 °N. However even within this restricted range, significant differences in size, condition and infection levels occurred between T.bubalis sampled from the coasts of Brittany, Devon, Cornwall and Wales. Populations were compared from the summer and autumn when maximum data was available. The summer sample included the newly recruited '0' group fish whilst that of the autumn the result of natural juvenile mortality and summer feeding. Although sea temperatures were thought to exert the greatest overall effect on determining the size, age and condition of the populations, the role played by genotype cannot be overlooked. In common with many other fish populations the breeding season becomes later towards the polar regions ensuring that the young are produced when sufficient food is available. The period of incubation is also likely to be temperature dependant as recorded by Swedmark (1958) for Pomatoschistus minutus, further securing the survival of the larvae. It was not possible to determine the exact spawning periods in the four regions but they would appear to agree with Cunningham (1891), Ehrenbaum (1904) and Fives (1970), ranging from January/February in southern Britain to March/April in the Baltic.

Size differences of fish between the regions were not always age dependant. The Roscoff sample, although one of the youngest was made up of significantly ($P<0.05$) larger fish than the other sites sampled in autumn. This could result from several factors including spawning time, availability of food and genotype. The Aberystwyth population however, sampled in the summer was both significantly larger and older than the other populations sampled then. The condition factor of the Roscoff population was not

significantly greater ($P<0.05$) than the other autumn samples, unlike that of Aberystwyth. This may be due to a number of factors including age and collection time. Sampling carried out at the beginning of the season in Roscoff was probably too early to record maximum condition factor, this having been shown in the Portwrinkle sample to proceed peak lipid percentage liver weight by one month. Nevertheless it was interesting to note that the Aberystwyth sample also exhibited the lowest incidence of hepatic parasites. As discussed earlier sex ratio increased with age in favour of the female and this was observed in all samples. The significantly lower ratio ($P<0.05$) observed at Widemouth may result from the lower nutrient intake due to the exposure of the site retarding growth and postponing maturation in the majority of males until their second year. Supporting the idea of lower intake are the significantly lower ($P<0.05$) values of both lipid percentage body weight and condition factor. The resting period of the gonads over the summer months made differences between the populations negligible. The variation in autumn represented different stages of maturity rather than potential fecundity. Large monthly samples throughout the autumn would be required to assess relative fecundity between the samples.

TRANSMISSION

Successful transmission of microsporidia will be influenced by spore viability, environmental conditions and host specificity and susceptability. Although it is accepted that infectivity is the only true test of viability, other criteria have been applied when the mode of transmission is

unknown. Integrity of the microsporidian spore and the extrusion of the polar filament by oxidizing agents such as iodine and hydrogen peroxide are features assumed to be indicative of viability here. On the basis of these criteria spores of Microgemma sp. are estimated to survive for at least 8 months in the marine environment. This is sufficient time for them to infect the succeeding generation of T.bubalis the following summer. That spores can survive long periods of time up to 10 years and maintain viability during this period has been demonstrated under laboratory conditions for two species infecting insects, namely Nosema apis and Nosema bombycis by Revelle (1960) and Oshima (1964) respectively. One of the factors contributing to their survival is probably the presence of chitin within the cell wall (Erickson 1967) which would afford adequate protection against many environmental factors, including dessication, bacterial infection and ultraviolet radiation (White 1919). Chitin is also likely to protect the spore passage through the digestive tract of most marine organisms preventing wastage by release in unfamiliar host. Temperature has also been found to influence survival and Oshima (1964) has shown that at lower temperature of 4^{o}C survival is greatly increased. In this respect it might be significant that the majority of spores of Microgemma sp. appear to be released in the autumn when they would gain from the lower winter temperatures enhancing survival until the following year. It was also noted here that spore survival period within the host was longer during winter than the summer. To what extent temperature affects this survival is not known, although it is likely that other temperature dependant factors such as the host immune response would be

involved in their destruction. Many spores that over winter in the host tissues are released during post spawning mortality of T.bubalis. The two main spore releasing periods within the life cycle of Microgemma sp. reflect the two periods of highest host mortality, the autumn period releasing spores chiefly from the 'O' group population and the winter period releasing spores from mature individuals. Whether spores are required to survive longer than 8 months is questionable. The well spaced spore releasing periods of autumn and spring suggest that 8 months is sufficient time for infection of the succeeding generation to occur. However the longer they survive the greater the chance of dispersal and the density of spores building up in the environment, which will increase the number of host contacts and enable survival in the event of a collapse in the host population. Results of this study indicate that juvenile fish are most susceptible to infection and it is probable that 'O' group T.bubalis are the most significant age group involved in transmission, the high infection levels probably contributing to mortality. Relatively low levels of infection recorded in groups 'I' and 'II' here could be explained by either protective immunity or change in diet. As appears typical for microsporidian parasites dependant on host mortality for transmission, ie Glugea stephani (Stunkard and Lux 1965, Takvorian and Cali 1981), Glugea hertwigi (Scarborough and Weidner 1979) and Tetramicra brevifilum (Matthews and Matthews 1981), the life cycle of Microgemma sp. is chiefly associated with disease in the juvenile population.

Once released into the marine environment it is advantageous

for spores to remain in the littoral zone where the chance of transmission is greatest. Floatation experiments here showed spores to be heavier than seawater, thus facilitating accumulation in rockpools. The continued location of spores within this habitat is assisted by the role of invertebrate rockpool fauna serving as transport or possibly intermediate hosts. Coastal turbulence may further influence the retention of spores in the littoral zone causing spores both free and within invertebrate hosts to be outwardly dispersed on exposed shores leading to a lower intensity of disease in the rockpool fish. Such factors would account for heavy infections being confined to the more sheltered aspects of the coast in Brittany on the South Finistaire coast 100% infection (B.F. Matthews pers.comm. 1982) rather than the northern site of Roscoff and on the south west Peninsula at Portwrinkle and Wembury rather than Widemouth. However it is likely that other factors cannot be ruled out.

The present study indicates Microgemma sp. to be a common parasite of T.bubalis within the latitudes of 48°N and 53°N. To what extent interchange of parasites occur via free spores or infective fish is unknown. Within the areas investigated the English Channel and to a lesser degree the Bristol Channel would provide formidable barriers to spore transfer between regions. It is unlikely that the spore alone will be transported across the channel since tidal streams tend to run laterally to the coast. This view is supported by recent epidemiological studies of the Haplosporidian Bonamia ostrea which despite annihilating much of the Breton and Dutch oyster

fishery in the last 10 years has failed to establish itself in Britain. A localised outbreak in Falmouth was attributed to the dumping of infected oysters from across the channel (Buck 1984). Microgemma sp. however unlike Bonamia ostrea which is restricted to mollusca incapable of extensive migrations, has a mobile fish host which may facilitate lateral and seaward dispersal of spores. While there is little evidence to suggest that T.bubalis undertakes such migration into deep water, the possibility that hepatic microsporidia might have a low degree of specificity to include offshore species should not be overlooked.

It is generally accepted that transmission of microsporidian spores within the aquatic environment involves transport hosts (Stunkard and Lux 1965, Putz and McLaughlin 1970, Olson 1975). A number of workers including Dykova and Lom (1978), Scarborough and Weidner (1972) and Olson (1975) have demonstrated that the addition of filter feeding crustacea to spore suspension greatly enhances the infection of fish. In the natural environment it would be equally important that spores could be recovered and concentrated before being presented to the fish host with food. If filter feeders serve as transport hosts, it seems unlikely that spores will be retained for any length of time in the gut without further development and it is possible that there is a continual turnover of spores in the benthic community. There is no evidence from experimental infections here to suggest that spores are transmitted directly by predation or cannibalism which further supports the view that spores require an environmental trigger, or

'priming' by passage through an invertebrate host (Weidner per.comm. Overstreet 1973). In looking for invertebrates which might possibly serve as transport hosts in the habitat of Taurulus bubalis a wide range of crustacea were commonly found both within the rockpool and in the stomach contents as listed under results. Harpacticoid copepods are here considered candidates to serve as transport hosts being scavengers and capable of handling spore sized particles of 2 - 4 µm. Not surprisingly however, in view of their small size, spores were not detected in samples of crustacea from rockpools during the present study. Experimental studies are recommended to confirm the involvement of these crustacea as transport hosts in transmission.

One of the objectives of the present study was to determine the degree of host specificity to Taurulus bubalis by Microgemma sp. and whether it could account for hepatic microsporidia in other marine teleosts including species of commercial importance. Hepatic microsporidiosis has been shown to be a common disease in marine teleosts in the area studied being recorded from Crenilabrus melops, Gaidropsarus mediterraneus, Ciliata mustela and Scopthalmus maximus in addition to T.bubalis. All these species were found in close proximity to T.bubalis, the wrasse and rockling in the same rockpools and the turbot on sand immediately adjacent to rocky outcrops supporting T.bubalis. Within the rockpool habitat it would be expected that all fish might be exposed to infection by the spores, differences in susceptibility accounted for by feeding preferences and innate characteristics. Structural studies of hepatic microsporidia from these rockpool hosts remains

inconclusive, however there is no evidence to date suggesting that they belong to a different species. Different degrees of microsporidian specificity have been recorded from marine teleosts. Glugea stephani shows a relatively low degree of specificity within the heterosomatous fishes being recorded from Pleuronectes platessa (Hägenmuller 1899), Pseudopleuronectes americanus (Woodcock 1904), Pleuronectes limanda (Reichenow 1953), Platyichthys stellatus (Jensen and Wellings 1972) and Limanda terruginea (Fantham, Porter & Richardson 1941). Taurulus bubalis, Myxcephalus scorpius and Lipophrys pholis on the other hand are all infected by different species of intramuscular Pleistophora, namely Pleistophora sp. investigated here, Pleistophora typicalis (Gurley 1893) and Pleistophora littoralis (Canning et al 1980). Feeding preferences may be partly responsible for this specificity, the fish infected with G.stephani all having similar dietary preferences of benthic molluscs and crustacea. In contrast T.bubalis, L.pholis and M.scorpius exploit different food - sources of M.scorpius being offshore and L.pholis showing a preference for barnacles. This dietary preference by L.pholis may also explain the absence of hepatic microsporidia. Feeding differences may also be responsible for the lower levels of hepatic microsporidiosis in the rockling, wrasse and turbot. Variation in the breeding season may result in some host species missing the main infection period and hence obtaining lower levels of infection. The main breeding period of T.bubalis is earlier than that of the rockling, wrasse and turbot (Russel 1935), and so presuming that the life cycle of Microgemma sp. is geared to T.bubalis the other potential hosts would miss the main

infective period. The parasites themselves may also influence specificity. Xenoma forming species such as Glugea may show a lower degree of specificity than say Pleistophora typicalis due to their manipulation of the host cell, suggesting that some of the parasite requirements are provided by itself. It is clear from the above discussion that factors determining specificity are not universal and depend to varying degrees on both the host and the parasite.

COURSE OF INFECTION

Release of Microgemma sp. into the external environment requires the death of the fish host Taurulus bubalis. The success of this mode of transmission is dependant on the high death rate in the young susceptible host population in which the majority of spores remain viable. This (naturally) high mortality in juvenile fish due to competition, starvation and predation may therefore enable the parasite even at sub lethal levels to be released. It is nevertheless probable, however, that Microgemma sp. will be a contributory factor to mortality, particularly at high infection levels, which is suggested by the decrease in mean infection levels.

Acute infection where merogony and sporogony are still active, mainly in summer and autumn, are confined to 'O' group and to a lesser extent 'I' group fish. The following chronic phase of the disease, characterised by the breakdown of the xenoma is found in all age groups, but is seldom found in 'O' group fish until the winter. This suggests that parasites can survive up to five months

before succumbing to immunological attack. That secondary infection might be an important aspect of microsporidian infection is suggested here from results showing active foci of infection in close proximity to old xenomas. As this was observed in winter it is possible that these originated from spores released on breakdown of xenomas. It is thought unlikely that active infections at this time would result from recent infection as ecological studies suggest that infection occurs between early summer and winter. Kudo (1966) previously recorded auto infection from several species of microsporidia and this may be considered a significant factor in increasing the potential of transmission by extending the life span of viable stages, particularly in winter when the host's immune system is less active due to the low temperatures.

The effect of microsporidiosis on marine and freshwater fish has been found to include decreased fecundity (Haley 1954, Sindermann 1963, Chen and Power 1972), growth retardation (Lom 1970, McVicar 1975), and mass mortality reviewed (Canning 1976). The effects of Microgemma sp. were less obvious as results were inconclusive in detecting measurable differences on host condition, liver weight, hepatic lipid content or gonad weight, although death was found to occur in cases of heavy infection in the laboratory. The results of good feeding conditions and optimum temperatures are believed to mask the effect of Microgemma sp. on condition factor, liver weight and lipid content of the liver. Condition factor, as discussed earlier is regarded as a long term measure of fitness resulting from the assimilation of food into body tissues and incorporating the effects of

stress. Results showed condition factor of T.bubalis not to be affected by infection levels of Microgemma sp. although it is thought that heavily infected fish prior to death must lose condition. The absence of this data supports the view that fish in poor condition do not survive long enough in the wild for examination. Under controlled conditions within the laboratory, Buckmann (1952) found that condition factor of Pleuronectes platessa was only affected by high levels of Glugea stephani together with conclusions drawn by MacKenzie (1981) on the insensitivity of condition factor with regard to hepatic coccidia in blue whiting, suggests that hepatic protozoans do not greatly interfere with host metabolic activities. It was expected however that the liver itself would be affected by the presence of Microgemma sp. and so liver percentage body weight was examined. From these results no significant change in weight or volume of the liver was incurred by level of infection, although it was difficult to obtain normal values of liver size as few uninfected controls were obtained. In addition there seemed to be no effect caused by the hemiurid metacercaria infection. That livers of fish can be directly affected by parasites has been demonstrated by the protozoan Eimeria sp. in blue whiting (Mackenzie 1981) and the nemátode Contracaecum aduncum in the Baltic cod, (Petrushevsky and Shulman 1958) which caused a reduction in liver weight with increase in infection levels. In mammals the increase in liver volume caused by Shistosoma sp. results from excessive fibrosis initiated by delayed hypersensivity reaction. Some workers have attempted to link the colour of the liver with condition (Kennedy 1976). However although the liver of T.bubalis varied between fish

there was no correlation with infection levels, age and seasonality being the most likely causes.

Total lipid percentage liver weight, considered to be an immediate indication of food consumption, again showed no interaction with infection levels, the overriding factor determining lipid content being season. It is thought likely however that Microgemma sp. may take advantage of the high hepatic lipid content in the late summer and autumn, which coincides with its greatest periodic activity. The apparent close relationship between hepatic lipid content and gonad maturation suggests that high infection levels with Microgemma sp. in late summer may utilize the lipid at the expense of host fecundity. Fecundity measured here as gonad percentage body weight however, did not show a significant interaction with Microgemma sp. Further investigations might look more closely at aspects of fecundity such as egg volume and increase sample sizes which may show some interaction.

From the present study evidence suggests that Microgemma sp. influences the longevity of T.bubalis, small numbers of older fish being detected with heavy infections and the lack of male fish in the upper age group. It is not a factor however accounting for the relatively short life span of the host Taurulus bubalis which has been shown by Lamp (1966) to be a natural phenomena in species collected outside the estimated apparent range of Microgemma sp. To what extent the survival of infected fish would be affected under conditions of environmental stress remains unknown, however this might become an important factor as a result of hepatic microsporidiosis in fish farms.

Although it would be expected that infection site within the liver would affect long term metabolic function of the liver including detoxification storage of nutrient reserves it would appear to have a less immediate effect on the host than infection of other sites such as the gut and muscle. Glugea stephani and Glugea hertwigi cause occlusion of the gut and mechanical damage to the intestine McVicar (1975) Olson (1976) and Legault and Delisle (1967) respectively, interfering with the passage and absorption of food. Tetramicra brevifilum (Matthews and Matthews 1981) affects the muscle of the fish and may cause liquifaction which will impede swimming and hence feeding and defence capabilities. The short lived nature of T.bubalis unlike that of the plaice, turbot or trout may facilitate transmission of Microgemma sp. by utilizing natural mortality. The only method of detecting the death of heavily infected fish in the environment is by recording the drop in numbers over the years. This approach was effectively used by Ziskowski and Murchelano (1975) to assess the effect of bacterial infections causing fin rot, suggesting that infection levels contribute to mortality. However until a laboratory system can be studied it is impossible to determine the cause of death in each case as dead or dying fish are rapidly removed from the 'wild population'.

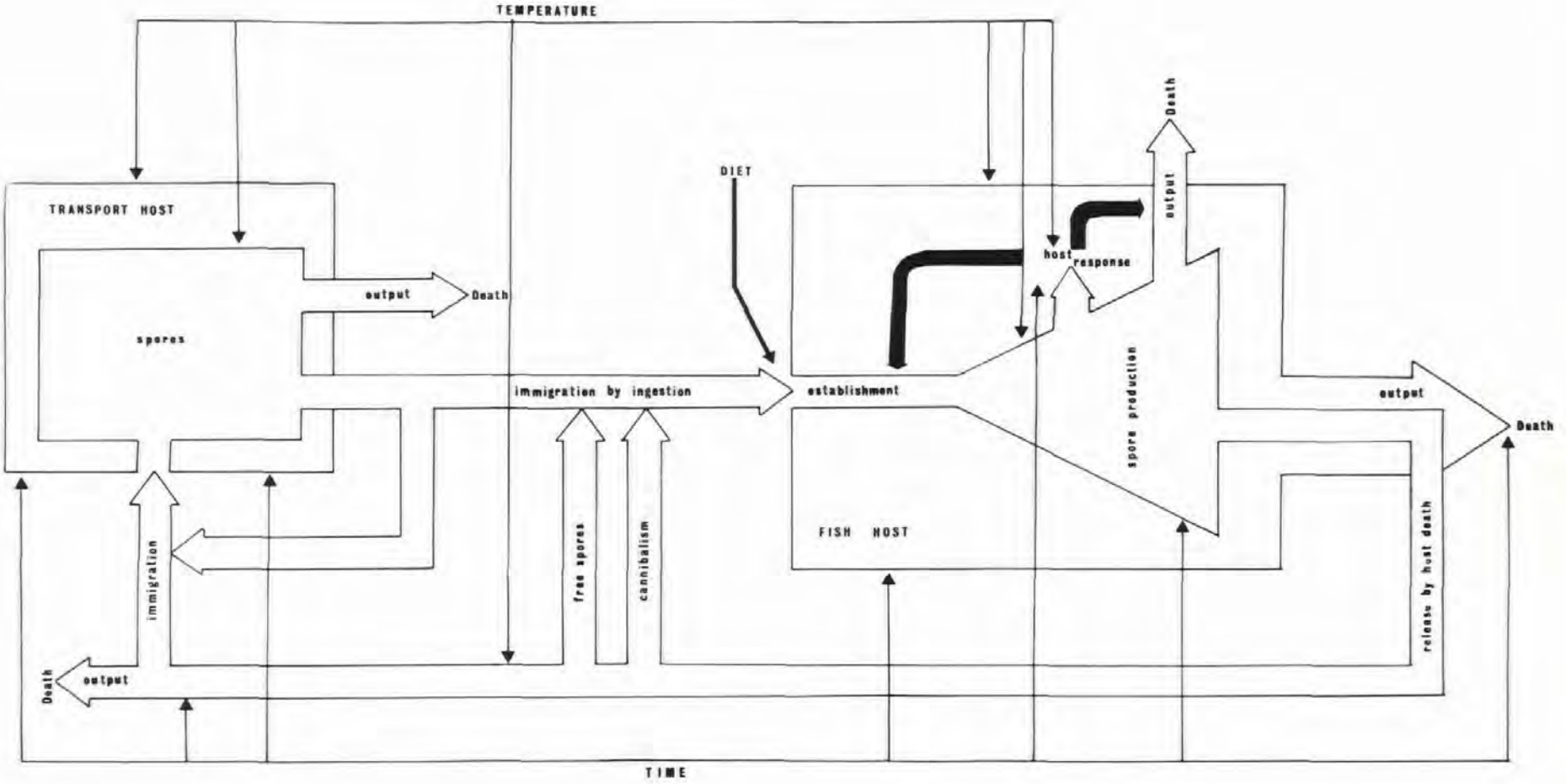
As mentioned earlier Microgemma sp. appears to occur within a fairly limited range of the host's distribution, northwards to Cardigan Bay and southwards towards Brittany, the highest incidence levels recorded at Portwrinkle. This northerly decline in incidence of Microgemma sp. implies that infection is dependant on environmental

conditions, notably temperature which is probably the most significant factor overall. The low sea temperatures around Scotland less than 15°C have already led McVicar (1975) to conclude that seas temperatures were responsible for the absence of Glugea stephani from wild populations there. Exposure too is thought to be a contributory factor in determining infection levels for reasons discussed earlier in transmission, the T.bubalis population at Widemouth supporting this. The presence of hemiurid metacercaria in the liver was investigated with respect to infection by Microgemma sp. and like the microsporidian, the metacercaria also requires the death of the host to continue their life cycle. Chi-square analysis showing T.bubalis infected with Microgemma sp. more likely to be infected with hepatic metacercaria than not, suggests the possibility of a common intermediate host, copepods being likely candidates as they are known to act as intermediate hosts for hemiurids and filter feeders have already been proposed as transport hosts by microsporidia (Stunkard and Lux 1965, Scarborough and Weidner 1979, McVicar 1975 and Olson 1976). The similar incidence levels of both parasites at Portwrinkle further support this.

MODELLING

The main aim of this project was to assess the possibility of constructing an epidemiological model and towards this end studies have been directed towards the identification of the parasite concerned and its geographical distribution within a specific range. Such a model will be dependant on a detailed knowledge of host parasite relations. Epidemiological models of parasitic diseases to date have been

Figure 93 Flow diagram presenting the life cycle of Microgemma sp. from Taurulus bubalis. (After Kennedy 1975).


TEMPERATURE
TRANSPORT HOST
spores
output
Death
DIET
host response
Death
output
immigration by ingestion
establishment
spore production
output
Death
FISH HOST
immigration
free spores
cannibalism
release by host death
Death
output
TIME

confined chiefly to medical parasitology notably malaria (McDonald 1957, Aron and May 1982) and shistosomiasis (Ansari 1973, Barbour 1982) where a great deal of data has been accumulated by many workers. The potential of epidemiological modelling in fish parasitology would be invaluable to the fish industry and in particular the fish farmer, enabling prediction of disease patterns. To date no studies have encompassed all epidemiological aspects of fish parasitology however many investigations including those by Summerfelt and Warner (1970), Pennycuick (1971 a,b,c) Chen and Power (1972) Scarborough and Weidner (1979) MacKenzie (1981) and Takvorian and Cali (1981) have gone some way towards it.

Although it is considered that insufficient data is available here to formulate a model it has nevertheless been possible to construct a flow diagram (fig.93). The value of such in order to assess the life cycle of Microgemma sp. from T.bubalis should not be underrated, for although the dynamics of a system can only be considered as a whole (Anderson 1976) it enables each component to be examined separately, and areas requiring further research to be identified and isolated. The data used in the flow diagram has been supplemented by studies on other species of fish microsporidia by McVicar (1975), Olson (1976), Dykova & Lom (1978), Matthews and Matthews (1980), Takvorian & Cali (1981).

In discussing the flow diagram in relation to both T.bubalis and Microgemma sp., it is convenient to consider first immigration of the spore and then establishment, growth and release. As infection occurs by oral ingestion it is assumed

that immigration is independant of previous or current parasitic burden (Anderson 1981). Exceptions to this assumption would be where parasitic burden changes the behavior of the host, a feature not recorded in the present study. Immigration is ultimately dependant on the density of spores in the immediate environment of T.bubalis and their concentration by transport hosts. It would also be affected by both environmental and host factors. The present study has already shown seasonality to affect the intensity of infection and it might be expected that immigration will be at its highest from from June to October when the fish population is at its highest and comprises of a large proportion of juveniles selectively feeding on crustacea. Cannibalism or predation of other infected fish species ie: Wrasse and Rockling may further affect the rate of immigration and could be a significant factor in the autumn when food and space become limited.

Assuming spores are viable, successful establishment and growth following immigration would be dependant on host susceptibility which may be determined by innate or acquired immunity. Within the range of T.bubalis from the Bay of Biscay to the polar seas one would expect genetic variation within the population, and in particular their ability to survive and breed at different temperatures. Apparent absence of hepatic microsporidia at the northerly end of the range could be explained on temperature differences although other factors such as genetic resistance may contribute. Delisle (1969), has suggested that physiological resistance could account for the absence of Glugea hertwigi in young of the year smelt from Green Lake, Quebec while causing heavy infection in smelt from Herey's Lake. Robertson (1979)

has shown that maturing male salmonids are more susceptible to Ichtyobodo necator than in mature or resting fish. This could help to explain the high levels of infection recorded from 'O' group male T.bubalis, which tend to mature in their first year. Growth of Microgemma sp.in T.bubalis does not appear to be interrupted by host reaction which was only seen to occur when sporolation had been completed.

Release of spores is dependant on host death which is facilitated particularly in juvenile T.bubalis by competition, starvation and predation, although undoubtably heavy parasitic burden will contribute as indicated by results. The timing of spore release is important as longevity of spores is estimated to be between 2 - 6 months, less than the natural life span of T.bubalis. Immigration of these spores into the transport host would be enhanced by the release of spores coinciding with high densities of transport hosts which is thought to occur in the autumn and late spring when fish host mortality is high.

The importance of the transport host in restricting spores within the fish habitat is not known, however those that do not enter transport hosts are likely to be carried away in offshore currents. The rockpool habitat will facilitate this stage of transmission by restricting both spore and host mobility. Although crustacea are considered to be the major transport hosts, fish may also be involved. It is not known whether transport hosts are scavengers, detritus or filter feeders, but spores are likely to pass through several such hosts, due to their short life span before reaching T.bubalis. Spores do not interact with the transport host and remain intact and viable. Those losing their

integrity by physical damage of seneisence will be susceptible to attack by digestive enzymes, bacterial or fungal infections. It is inevitable that some spores will end up in non-preferred fish hosts and although they may establish themselves and reproduce they are unlikely to be released to continue their life cycle.

Sea temperatures within the area studied are not subject to wide fluctuations, mean inshore levels around Portwrinkle ranging from 6.5°C in January to 17.8°C in September. Rockpool temperatures however will show greater variation (Stubbs 1980). Microgemma sp. will be exposed directly to temperature fluctuation being either free in the environment or within poikilothermic hosts. Rates of immigration, establishment, spore production and release are not thought to be affected by the above temperature ranges. Provided the intensity of spores in the environment and transport hosts is high, temperature of 16°C plus may increase immigration by promoting more active feeding responses to support the higher metabolic rate. The apparent absence of Microgemma sp. in T.bubalis north of Aberystwyth suggests that infection is limited by low temperatures. The absence of Glugea stephani in plaice off the coast of Scotland according to McVicar (1975) results from the low sea temperatures. Rapid increase in infection levels over the summer and early autumn support the idea that high temperatures increase the rate of parasitic development, however it may also elevate host's immune response. The lowering of immunological constraint on the parasite by the host during the low winter temperatures may allow auto-infection to occur again increasing the level of infection. The release

of spores is indirectly affected by temperature, high parasitic burdens and host response during the high summer temperatures contributing to host death.

There appears to be no reason why spores cannot survive several seasons in the environment as laboratory experiments show microsporidian spores of insect hosts remain viable for up to 10 years (Oshima 1964). This would ensure survival of the parasite in the event of a host population collapse. Age of the host may effect immigration by change in feeding habitats and acquired immunity. In order to release the maximum number of viable spores, the timing of host death is important. It seems unlikely therefore that death by ageing of the host contributes significantly to the spore pool as by this time host reaction has probably rendered the majority of spores inviable. The parasite is thought to utilize the naturally high rates of juvenile and post spawning mortality to facilitate release, although parasitic burden undoubtably contributes.

Many areas of research remain unresolved in this project and many come to light in the examination of host parasite relations. Problems requiring urgent attention include those of host specificity and transmission. A successful laboratory based experimental system backed up by ultrastructural studies of developmental stages of all hepatic species would enable these areas to be investigated.

TAXONOMY

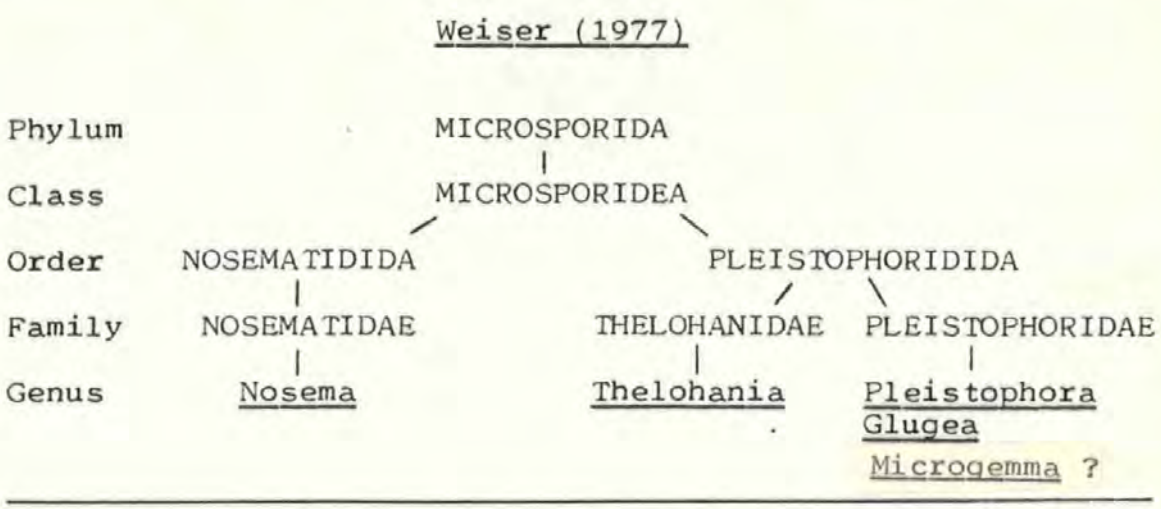
Microgemma sp. is here considered to be synonomous with Pleistophora typicalis, recorded from the liver of the same host namely Taurulus bubalis by Dunkerly (1920) and is referred to from hereon as Microgemma dunkerli.

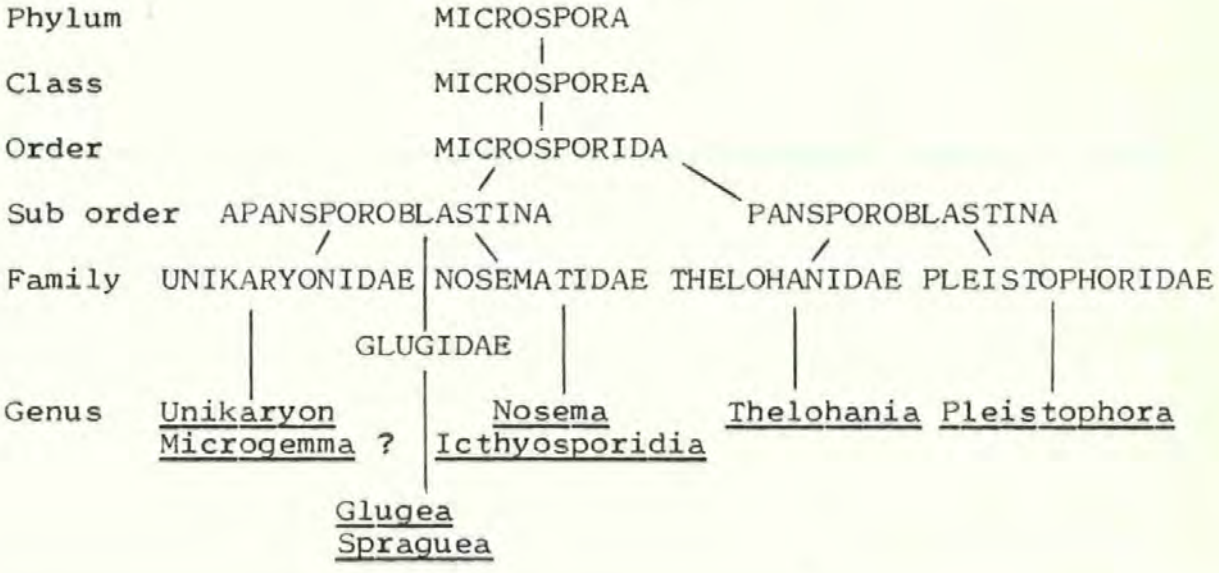
Pleistophora typicalis was first recorded by Thélohan (1891) from the musculature of Myxcephalus scorpius and later from that of Taurulus bubalis, Lipophrys pholis and Pungitus pungitus (Thélohan 1895). Pleistophora typicalis from the host type Myxcephalus scorpius has recently been described by Canning and Nicholas (1980), and its inclusion within Pleistophora confirmed. Further studies of P.typicalis from Lipophrys pholis (Canning, Hazard and Nicholas 1979) has since shown this to be a separate species namely Pleistophora littoralis. It now seems probable that in the light of these investigations further research will cast doubt on the validity of P.typicalis as a microsporidian with a low degree of specificity. The identity of intramuscular species from T.bubalis is now in question and although this study confirms it as Pleistophora sp., further studies will be required to distinguish specific features. It was noticeable here however that it differed from P.typicalis and P.littoralis by the number of coils of polar filament which were constantly less than 10, contrary to the former were always in excess of 10. Despite this notable difference it is considered here that there is insufficient evidence to justify a specific name and it is therefore advised that this be regarded as Pleistophora sp. One feature that might prove significant however would be the presence of macrospores which could be revealed in a more detailed investigation. Studies here show that two new species of microsporidia commonly occur in T.bubalis namely Microgemma dunkerli and Pleistophora sp.

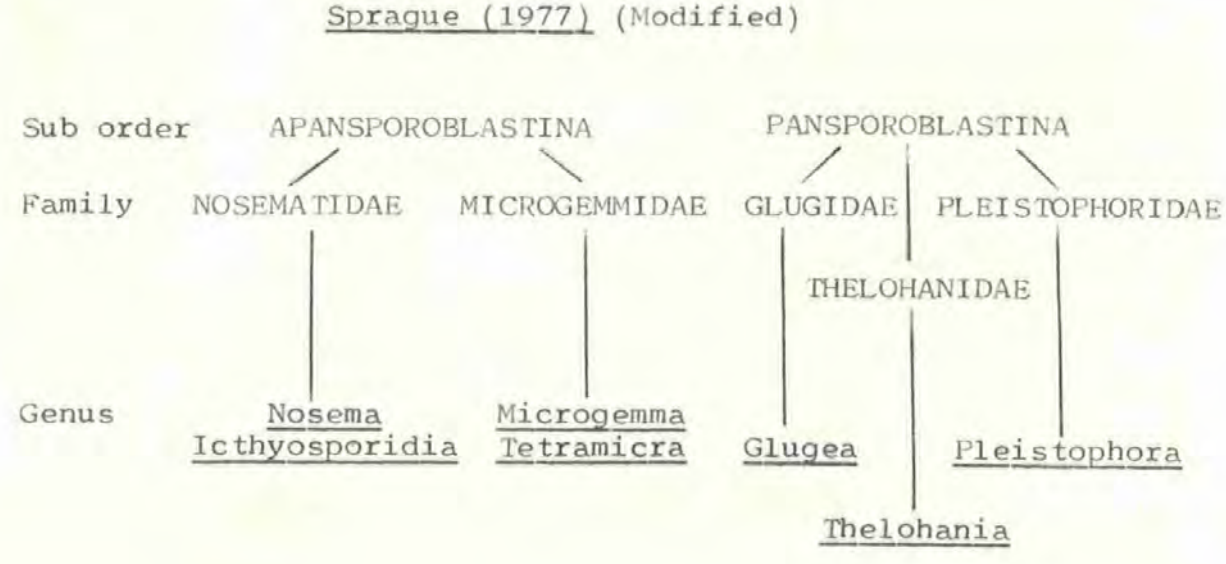
In further classification of Microgemma dunkerli it has been necessary to review current literature on taxonomy. The two most recent schemes have been put forward by Sprague (1977)

and Weiser (1977), who both agreed that the group should be elevated to Phylum, Microsporidia being retained by Weiser and Microspora introduced by Sprague. The species recorded from fish are confined to the orders Nosematidida (Laabé 1899) and Pleistophoridida (Stempell 1909) by Weiser and the single order Microsporida (Balbiani 1882) by Sprague. The following diagram (fig.94) illustrates the relationships between the relevant sections of schemes proposed by Weiser (1977) and Sprague (1977) together with a new scheme based on Sprague (1977). The genera Microgemma does not fall clearly into any of the families proposed by Sprague (1977) although it clearly belongs to the sub order Apansporoblastina (Tuzet et al 1971). The absence of a pansporoblastic membrane automatically excludes it from the Pleistophoridae (Tuzet et al 1971) and Thelohanidae (Hazard and Oldacre 1975), the only families within the sub order Pansporoblastina to include species from fish hosts. Within the sub order Apansporoblastina again only two families namely Glugidae (Thélohan 1895) and Nosematidae (Laabé 1899) include species infecting fish and although the genus Microgemma shows some similarities with these families there are sufficient differences to exclude it from both. It clearly differs from the description of Glugidae by the absence of a sporogonic vacuole and its polysporoblastic development. However Canning et al (1982) have redefined Glugea (Thélohan 1891) as multisporous and pansporoblastic, which may result in the family Glugidae being split accordingly. Unikaryonidae is the only family proposed by Sprague (1977) which could accommodate Microgemma sp. although this is probably due to the characteristics being less specific than for other families in the sub order

Figure 94 Comparison of taxonomic schemes for Microgemma sp.







allowing greater flexibility in the species included. The species currently in this family appear to be specialised hyperparasites of trematodes and gregarines making a case for the erection of a new family to include species from fish hosts.

The taxonomic scheme of Weiser (1977) places the genera Glugea and Pleistophora together within the same family Pleistophoridae (Stempell 1909), characteristic features being asynchronous polysporoblastic development, the presence of uninucleate sporoblasts and spores and monomorphism. Microgemma would be included in this family as it now stands. Recent investigations however (Canning, Hazard and Nicholas 1979, Canning and Nicholas 1980) have shown Pleistophora to include dimorphic genera and if this feature is considered to be a family characteristic the Pleistophoridae (Stempell 1909) requires division. As with most taxonomic schemes, both classifications are in need of reorganization in the light of recent investigations here and by others. In the present investigation the scheme proposed by Sprague (1977) is favoured as it is thought to indicate more clearly the taxonomic relationships of Microgemma dunkerli with other groups.

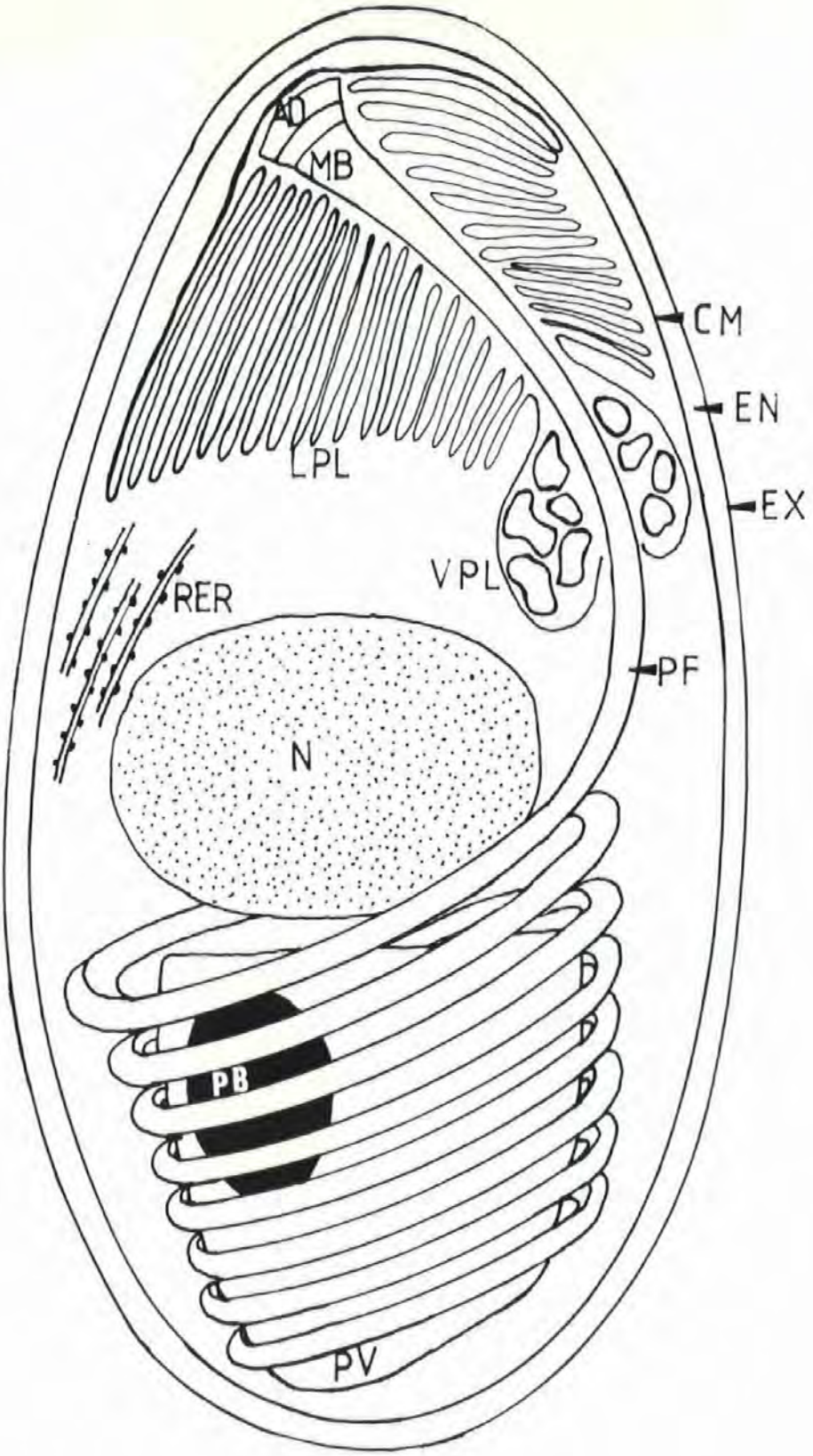
The sub orders Apansporoblastina and Pansporoblastina are upheld at present, although Canning and Vavra (1977) doubt the need for such a division as dimorphic species have shown both types of development. In this study the sub order Apansporoblastina would include all species which develop in direct contact with the host cell cytoplasm or partly within a host derived membrane, whereas the sub order Pansporoblastina would include all species which develop

partly or wholly within a parasitophorous vacuole at some stage in their life. The reclassification of Glugea by Canning, Hazard and Nicholas (1979) as mentioned previously has required its removal to the sub order Pansporoblastina. This further separates the genera Glugea and Microgemma and as a result the erection of a new family Microgemmidae is proposed to include Microgemma hepaticus, Microgemma dunkerli and Tetramicra brevifilum. The latter species is included at the expense of the recently erected family Tetramicridae (Matthews and Matthews 1981). Characteristics of Microgemmidae are listed below.

Studies of other species of microsporidia suggest that the site of infection is often an important taxonomic feature. This may occur at generic level, ie Loma sp. Morrison and Sprague 1981) from the gill lamellae, or at specific level, ie Glugea cotti (Chatton and Courrier 1923) from the testis and Tetramicra brevifilum (Matthews and Matthews 1981) from the perimysium of muscle tissue. Microgemma is here considered to be a site specific genus since both Microgemma dunkerli and Microgemma hepaticus are exclusive to the liver. Ultrastructural studies suggest that in the case of Microgemma sp. from T.bubalis the target cell is a hepatocyte rather than a migratory or endothelial cell. Species other than these which have been recorded solely from the liver of teleosts in this study namely Gaidropsarus mediterraneus, Ciliata mustela, Scopthalmus maximus and Crenilabrus melops will be included within the genus Microgemma, although further studies are required to determine whether they are synonomous with Microgemma dunkerli or Microgemma hepaticus. Hepatic microsporidia from outside these waters, notably Nosema ovoideum from Mullus barbatus

(Raabe 1936) and Glugea ovoideum (Thélohan 1895) from Cepola rubescens recorded from the mediterranean may also be included in the genus Microgemma. Previously Sprague (1977) relegated these species to the genera Microsporidium because of their brief descriptions, which although short show characteristics of the genus Microgemma, including exclusive location within the liver, xenoma formation and a similar host type. Other microsporidian species recorded exclusively from the liver are Glugea depressa (Thélohan 1895) and Glugea machari (Jírovec 1934). The description of the former species which occurs in Julis vulgaris does not exclude it from the genus Microgemma, although further studies are required to supplement its brief description before it can be conclusively classified. Glugea machari on the other hand is found in the superficial layers of the liver which together with the spore description appear to exclude it from the genus Microgemma. Icthyosporidium giganteum (Thélohan 1895, Swarczewsky, 1914) reported from the body cavity of the wrasse by many authors was recorded from the liver of Crenilabrus melops by Le Danois (1910). This species however was clearly distinguishable from Microgemma dunkerli and the hepatic microsporidia found in C.melops from this study by its xenoma, spore structure and sporogony. Although there is a possibility of hepatic microsporidia being attributed to one species, as slight structural differences could be dependant on host factors, it is considered here that only by transmission can the specificity of Microgemma sp. be determined. Other members of the genus Cottus, namely Cottus gobio and Cottus beldingii are infected with abdominal intramuscular Pleistophora, the former with P.vermiformis and the latter by

Figure 95 Spore of Microgemma dunkerli. Diagrammic representation to show characteristic features, notably the single nucleus, 7 coils of polar filament surrounding the posterior vacuole and the presence of an inclusion body.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P. tahoensis (Summerfelt and Ebert 1969). Since comparative parasitology shows host specificity to be a function of evolutionary age and specialisation (Cameron 1952) this host specific relationship between P. vermiformis and C. gobio, and P. tahoensis and C. beldingii is considered to be older than that of Cottus sp. and Pleistophora sp. (Wales and Wolf 1955). Cottus sp. appears to serve as a secondary or recipient host, to the primary Salmonid hosts. The coincidence of two Pleistophorans affecting the same site in fresh water cottids from opposite sides of the Atlantic suggests evolution from a common ancestor which evolved in the North Pacific region (Sandercock and Wilmousky 1968).

Characteristics of Microgemma dunkerli sp. n.

Microgemma dunkerli sp. n.

Type host Taurulus bubalis (Euphr.)

Locality S.W. Britain, Brittany.

Uninucleate spores (4.6 x 2.3 µm) characterized by 7 - 10 coils of isofilar polar filament (approximately 53 µm extruded) and a posterior vacuole with inclusion body (fig.95). Merogony of uninucleate and multinucleate plasmodia enveloped in host cell membrane. Asynchronous sporogony giving rise to 2 - 6 mother sporoblast cells by repeated budding from which further division into two occurs prior to sporoblast development. Sporogony is characterized by the laying down of an electron dense coat. Xenoma (35-750 µm diameter) with obvious zonation of reticulated host nuclei and developmental parasitic stages.

Microgemmidae Aplansporoblastic development. Polysporoblastic sporogony in direct contact with host cell cytoplasm. Uninucleate spore and sporoblast. Specific to teleost hosts.

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A P P E N D I X 1

Site: PORTWRINKLE

Data sheet No. 1.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
5	T.bub.	22.01.81	5.8	3.0	1.54	0	-1	-1	-1	-1	-1	-1	-1	3	3
6	C.mus.	22.01.81	10.4	9.0	0.80	-1	-1	-1	-1	-1	-1	-1	-1	1	1
7	L.pho.	22.01.81	10.0	10.0	1.00	-1	2	-1	-1	-1	-1	-1	-1	0	0
8	G.fla.	22.01.81	4.0	1.0	1.56	-1	2	-1	-1	-1	-1	-1	-1	0	0
9	T.bub.	22.01.81	6.8	6.0	1.91	0	-1	-1	-1	-1	-1	-1	-1	2	1
10	T.bub.	22.01.81	8.5	18.0	2.93	1	2	-1	-1	-1	-1	-1	-1	0	1
11	C.lyr.	22.01.81	8.0	5.0	0.98	-1	-1	-1	-1	-1	-1	-1	-1	0	0
12	T.bub.	22.01.81	11.0	29.0	2.18	2	2	-1	-1	-1	-1	-1	-1	0	1
13	T.bub.	22.01.81	11.0	26.0	1.95	2	2	-1	-1	-1	-1	-1	-1	0	0
14	T.bub.	22.01.81	6.2	5.0	2.10	0	-1	-1	-1	-1	-1	-1	-1	3	1
15	T.bub.	22.01.81	9.6	23.0	2.60	2	2	-1	-1	-1	-1	-1	-1	0	0
16	T.bub.	22.01.81	6.5	5.0	1.82	0	-1	-1	-1	-1	-1	-1	-1	2	2
17	T.bub.	22.01.81	7.0	7.0	2.04	1	-1	-1	-1	-1	-1	-1	-1	2	2
18	T.bub.	22.01.81	6.8	6.0	1.91	0	-1	-1	-1	-1	-1	-1	-1	2	2
19	T.bub.	22.01.81	6.2	4.5	1.89	0	-1	-1	-1	-1	-1	-1	-1	3	2
20	T.bub.	22.01.81	6.5	5.5	2.00	1	1	-1	-1	-1	-1	-1	-1	2	1
32	T.bub.	03.04.81	7.3	6.4	1.65	1	-1	-1	-1	-1	-1	-1	-1	0	2

Site: PORTWRINKLE

Data sheet No. 2.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
33	T.bub.	03.04.81	7.8	7.2	1.52	1	-1	-1	-1	-1	-1	-1	-1	0	2
34	T.bub.	03.04.81	8.0	8.1	1.58	1	1	-1	-1	-1	-1	-1	-1	0	1
35	T.bub.	03.04.81	7.5	6.0	1.42	1	1	-1	-1	-1	-1	-1	-1	3	1
41	T.bub.	21.05.81	9.5	14.5	1.69	2	1	-1	-1	-1	-1	-1	-1	0	1
42	T.bub.	21.05.81	7.5	6.3	1.49	1	1	-1	-1	-1	-1	-1	-1	2	2
43	C.mus.	21.05.81	10.5	10.0	0.86	-1	1	-1	-1	-1	-1	-1	-1	2	1
44	C.mus.	21.05.81	15.0	23.3	0.69	-1	2	-1	-1	0.42	1.80	-1	-1	0	1
68	T.bub.	14.06.81	8.5	11.6	1.89	1	2	-1	-1	-1	-1	-1	-1	3	1
69	C.mus.	14.06.81	11.0	8.5	0.64	-1	-1	-1	-1	-1	-1	-1	-1	0	0
70	C.mus.	14.06.81	04.0	3.5	5.47	-1	0	-1	-1	-1	-1	-1	-1	0	0
71	G.med.	14.06.81	14.5	21.0	0.69	-1	2	-1	-1	-1	-1	-1	-1	2	1
74	C.mus.	07.07.81	3.6	0.2	0.43	-1	-1	-1	-1	-1	-1	-1	-1	0	0
76	P.pla.	09.07.81	-1	-1	-1	-1	0	-1	-1	-1	-1	-1	-1	0	0
77	C.mus.	13.07.81	6.5	1.7	0.62	-1	-1	-1	-1	-1	-1	-1	-1	0	1
78	T.bub.	13.07.81	7.5	11.6	2.75	1	1	-1	-1	-1	-1	-1	-1	1	2
79	T.bub.	13.07.81	3.1	0.7	2.35	0	-1	-1	-1	-1	-1	-1	-1	0	0
80	T.bub.	13.07.81	3.4	0.9	2.16	0	-1	-1	-1	-1	-1	-1	-1	2	1

Site: PORTWRINKLE Data sheet No. 3.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
81	T.bub.	13.07.81	4.0	1.3	2.03	0	-1	-1	-1	-1	-1	-1	-1	0	1
82	T.bub.	13.07.81	3.8	1.2	2.19	0	-1	-1	-1	-1	-1	-1	-1	0	1
83	T.bub.	13.07.81	3.0	0.6	2.22	0	-1	-1	-1	-1	-1	-1	-1	0	0
84	T.bub.	13.07.81	2.7	0.4	2.03	0	-1	-1	-1	-1	-1	-1	-1	0	1
102	T.bub.	01.09.81	5.0	4.2	3.36	0	-1	-1	-1	-1	-1	-1	-1	1	3
103	T.bub.	01.09.81	4.5	2.5	2.74	0	-1	-1	-1	-1	-1	-1	-1	2	3
104	T.bub.	01.09.81	3.5	1.7	3.97	0	-1	-1	-1	-1	-1	-1	-1	0	2
105	T.bub.	01.09.81	9.4	19.0	2.29	2	2	-1	-1	-1	-1	-1	-1	2	2
106	C.lyr.	01.09.81	7.0	4.6	1.34	-1	-1	-1	-1	-1	-1	-1	-1	0	1
107	G.fla.	01.09.81	3.5	0.6	1.40	-1	-1	-1	-1	-1	-1	-1	-1	0	0
108	L.pho.	01.09.81	9.0	10.3	1.41	-1	2	-1	-1	-1	-1	-1	-1	0	2
109	G.nig.	01.09.81	5.4	2.8	1.78	-1	-1	-1	-1	-1	-1	-1	-1	1	0
120	T.bub.	13.10.81	6.0	6.8	3.15	1	-1	-1	-1	-1	-1	-1	-1	2	0
121	T.bub.	13.10.81	4.5	2.6	2.85	0	2	-1	-1	-1	-1	-1	-1	1	3
122	T.bub.	13.10.81	7.5	10.5	2.49	1	1	-1	-1	-1	-1	-1	-1	1	1
123	T.bub.	13.10.81	8.1	19.1	2.90	1	2	-1	-1	-1	-1	-1	-1	0	1
124	T.bub.	13.10.81	9.2	18.2	2.34	1	2	-1	-1	-1	-1	-1	-1	1	1

Site: PORTWRINKLE

Data sheet No. 4.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
125	T.bub.	13.10.81	12.0	43.4	2.31	2	1	-1	-1	-1	-1	-1	-1	1	1
126	T.bub.	13.10.81	5.0	2.9	2.32	0	1	-1	-1	-1	-1	-1	-1	3	1
127	C.mus.	13.10.81	17.5	52.3	0.06	-1	2	-1	-1	-1	-1	-1	-1	2	0
128	T.bub.	13.10.81	8.0	15.3	2.99	1	1	-1	-1	-1	-1	-1	-1	2	0
129	T.bub.	13.10.81	9.0	17.7	2.43	1	1	-1	-1	-1	-1	-1	-1	0	2
130	T.bub.	13.10.81	8.5	18.6	3.03	1	2	-1	-1	-1	-1	-1	-1	2	0
131	G.med.	13.10.81	16.0	42.0	1.03	-1	2	-1	-1	-1	-1	-1	-1	1	0
132	G.med.	13.10.81	16.0	29.2	0.71	-1	2	-1	-1	-1	-1	-1	-1	0	0
133	C.mel.	13.10.81	4.3	1.3	1.64	-1	-1	-1	-1	-1	-1	-1	-1	0	0
134	T.bub.	22.10.81	2.0	0.3	3.75	0	-1	-1	-1	-1	-1	-1	-1	0	3
135	T.bub.	22.10.81	2.0	0.2	3.75	0	-1	-1	-1	-1	-1	-1	-1	1	3
146	T.bub.	17.11.81	8.2	17.0	3.08	1	1	0.0500	0.290	0.2400	1.47	-1	-1	1	0
149	T.bub.	17.11.81	4.0	1.4	2.19	0	1	0.0100	0.710	-1	-1	-1	-1	3	2
150	T.bub.	01.12.81	6.5	6.8	2.48	0	2	0	0	0.1000	1.47	-1	-1	3	2
151	T.bub.	01.12.81	9.0	7.9	1.08	1	2	0.3000	3.330	0.4000	5.06	-1	-1	1	2
152	T.bub.	01.12.81	7.0	8.5	2.48	1	2	0.0900	1.060	-1	-1	-1	-1	2	3
166	T.bub.	14.12.81	5.0	3.5	2.80	0	1	0	0	0.0900	2.57	-1	-1	3	3

Site: PORTWRINKLE Data sheet No. 5.

Case No.	Species	Date	Length cm	Width grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met.	Infection Mic.
167	T.bub.	14.12.81	8.8	18.1	2.66	1	2	0.9000	4.970	1.0700	5.90	0.0143	1.336	1	1
168	T.bub.	14.12.81	8.3	15.6	2.73	1	2	0.1000	7.050	1.2200	7.80	0.0137	1.12	2	1
169	T.bub.	14.12.81	11.0	46.4	3.49	2	2	1.9100	4.120	2.4300	5.20	0.0715	2.9423	1	0
171	T.bub.	14.12.81	10.4	30.4	2.70	2	2	3.3000	10.800	0.4600	1.50	-1	-1	2	2
172	T.bub.	14.12.81	8.0	18.1	3.54	1	1	0.6300	3.480	0.4500	2.50	0.00396	8.8	2	0
173	T.bub.	14.12.81	11.3	42.6	2.95	2	2	0.8700	2.040	1.0600	2.50	-1	-1	1	0
174	T.bub.	14.12.81	5.0	3.3	2.64	0	1	0	0	0.0500	1.50	0.00068	1.36	1	3
175	T.bub.	14.12.81	5.1	6.3	4.75	0	1	0	0	0.1000	1.60	0.00046	0.46	1	3
176	T.bub.	14.12.81	4.0	1.7	2.66	0	2	0	0	0.0200	1.20	-1	-1	1	3
177	T.bub.	14.12.81	5.4	3.9	2.48	0	2	0	0	0.0500	1.20	-1	-1	2	1
178	T.bub.	14.12.81	5.0	3.4	2.72	0	2	0	0	0.0300	0.80	-1	-1	1	2
179	T.bub.	14.12.81	5.7	4.7	2.54	0	1	0	0	0.1000	2.00	-1	-1	2	2
180	T.bub.	14.12.81	5.1	3.7	2.79	0	1	0	0	0.0700	1.90	-1	-1	2	3
181	T.bub.	14.12.81	4.8	2.8	2.53	0	2	0	0	0.0500	1.80	-1	-1	3	2
182	T.bub.	14.12.81	5.0	2.9	2.32	0	2	0.0300	01.030	0.0500	1.70	0.00069	1.38	2	3
183	T.bub.	14.12.81	5.0	3.1	2.48	0	1	0	0	0.0300	0.90	0.00010	0.33	2	1
184	T.bub.	14.12.81	4.0	1.6	2.50	0	1	0	0	0.0200	1.30	0.00031	1.55	2	3

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Data sheet No. 6.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
185	C.mel.	10.01.82	3.9	0.9	1.51	-1	-1	-1	-1	-1	-1	-1	-1	0	2
186	T.bub.	14.12.81	4.4	1.7	2.00	0	2	0	0	0.0200	1.17	0.0007	3.5	2	3
187	T.bub.	09.02.82	5.9	5.5	2.68	0	1	0	0	0.1200	2.18	-1	-1	3	1
188	T.bub.	09.02.82	5.0	3.5	2.80	0	2	0	0	0.0400	1.14	-1	-1	3	0
190	C.mel.	09.02.82	5.2	2.7	1.92	-1	-1	-1	-1	-1	-1	-1	-1	0	0
191	T.bub.	09.02.82	3.7	1.6	3.20	0	2	0	0	0.0900	5.6	-1	-1	0	0
192	T.bub.	09.02.82	5.8	5.9	3.00	0	2	0	0	0.1500	2.54	-1	-1	3	0
193	T.bub.	09.02.82	5.4	5.2	3.30	0	1	0	0	0.0800	1.54	-1	-1	2	2
194	T.bub.	09.02.82	4.5	2.6	2.85	0	1	0	0	0.0250	1.96	-1	-1	3	1
195	T.bub.	09.02.82	5.8	4.1	2.40	0	1	0.0150	0.3600	0.0700	1.70	-1	-1	2	3
196	T.bub.	09.02.82	5.5	5.2	3.13	0	1	0	0	0.0800	1.50	-1	-1	3	2
198	G.med.	09.02.82	19.0	75.5	1.10	-1	2	0.5200	0.6887	3.2800	4.34	-1	-1	2	0
199	T.bub.	09.02.82	4.8	3.6	3.25	0	1	0	0	0.0400	1.10	-1	-1	3	3
200	G.med.	01.03.82	14.0	25.5	0.93	-1	2	-1	-1	0.1280	0.50	-1	-1	0	1
201	T.bub.	09.02.82	3.8	1.5	2.73	0	1	0	0	0.0340	2.26	-1	-1	0	2
202	T.bub.	09.02.82	5.1	3.8	2.86	0	1	0	0	0.0929	2.44	-1	-1	3	2
203	T.bub.	09.02.82	5.2	4.6	3.27	0	2	0.0146	0.3170	0.1293	2.81	-1	-1	2	2

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Data sheet No. 7.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
204	T.bub.	09.02.82	8.8	18.2	2.67	1	2	0.2892	1.5890	0.3895	2.14	-1	-1	2	2
205	T.bub.	09.02.82	4.5	2.6	2.85	0	2	0	0	0.0674	2.20	-1	-1	3	2
206	T.bub.	09.02.82	5.8	5.3	2.72	0	1	0	0	0.1134	2.22	-1	-1	1	2
208	P.gat.	09.03.82	4.0	1.5	2.34	-1	-1	-1	-1	-1	-1	-1	-1	0	0
209	T.bub.	09.03.82	5.5	5.2	3.13	0	2	0.0169	0.3170	0.1178	3.02	-1	-1	1	3
210	T.bub.	09.03.82	6.1	7.2	3.15	1	1	0.3180	4.4470	0.1105	2.12	-1	-1	1	3
211	T.bub.	09.03.82	4.5	2.1	2.30	0	1	0	0	0.0429	2.04	-1	-1	2	3
212	T.bub.	09.03.82	6.0	5.8	2.68	0	1	0	0	0.1245	2.14	-1	-1	2	3
213	T.bub.	09.03.82	6.1	7.1	3.12	0	2	0	0	0.1567	2.20	-1	-1	1	1
214	T.bub.	09.03.82	8.5	17.6	2.87	1	2	1.9928	11.320	0.3917	2.22	-1	-1	1	1
215	T.bub.	09.03.82	6.1	6.1	2.69	0	2	0	0	0.1409	2.31	-1	-1	2	1
216	C.mus.	09.03.82	8.4	5.3	0.89	1	2	-1	-1	0.1230	2.32	-1	-1	1	2
217	T.bub.	09.03.82	5.5	4.3	2.59	0	2	0.0147	0.3420	0.0916	2.13	-1	-1	3	2
218	T.bub.	09.03.82	6.1	5.5	2.42	0	2	0.0120	0.2180	0.1049	1.90	-1	-1	0	1
219	T.bub.	09.03.82	5.9	5.0	2.43	0	2	0.0150	0.3000	0.0947	1.89	-1	-1	1	2
220	T.bub.	09.03.82	6.7	9.2	3.05	1	2	1.3100	14.227	0.5192	5.64	-1	-1	2	3
229	T.bub.	26.04.82	5.0	3.4	2.72	1	1	0	0	0.0980	2.88	-1	-1	2	2

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Data sheet No. 8.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
230	T.bub.	26.04.82	5.6	5.5	3.13	1	1	0	0	0.1725	3.14	-1	-1	1	1
231	L.pho.	26.04.82	5.6	2.7	1.54	-1	2	-1	-1	-1	-1	-1	-1	0	0
233	T.bub.	26.04.82	5.8	6.1	3.13	1	2	-1	-1	0.0201	0.33	-1	-1	0	2
234	T.bub.	26.04.82	7.2	10.5	2.81	1	2	0.0246	0.2343	0.1660	1.58	-1	-1	2	3
235	T.bub.	26.04.82	5.1	4.4	3.32	1	2	0.0141	0.3205	0.0763	1.73	-1	-1	1	3
236	C.mel.	26.04.82	6.5	6.1	2.22	-1	-1	-1	-1	-1	-1	-1	-1	0	1
237	G.med.	26.04.82	6.4	44.2	1.00	-1	2	-1	-1	-1	-1	-1	-1	1	1
238	T.bub.	26.04.82	6.5	8.4	3.06	1	1	0.0405	0.4821	0.1959	2.33	-1	-1	3	3
239	T.bub.	26.04.82	7.5	22.2	5.26	1	2	0.0445	0.2004	0.2570	1.16	-1	-1	1	2
240	C.mel.	26.04.82	5.4	3.9	2.48	-1	-1	-1	-1	-1	-1	-1	-1	2	0
241	C.mel.	26.04.82	6.5	5.6	2.04	-1	-1	-1	-1	-1	-1	-1	-1	0	1
242	C.mus.	26.04.82	14.1	21.7	0.77	-1	-1	-1	-1	-1	-1	-1	-1	0	2
243	C.mus.	26.04.82	10.0	10.4	1.04	-1	2	-1	-1	-1	-1	-1	-1	2	0
244	T.bub.	26.04.82	7.0	10.6	3.09	1	2	0.284	0.2679	0.1873	1.77	-1	-1	2	2
245	L.pho.	26.04.82	9.0	10.5	1.44	-1	-1	-1	-1	-1	-1	-1	-1	0	0
246	T.bub.	27.05.82	7.0	10.0	2.92	1	1	0.0086	0.0860	0.2100	2.10	.00154	0.73	0	1
247	T.bub.	27.05.82	8.5	16.4	2.67	2	1	0.0700	0.4268	0.2818	1.72	.00124	0.39	2	2

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Data sheet No. 9.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
248	T.bub.	27.05.82	5.9	6.6	3.21	1	1	0.0050	0.0758	0.1759	2.66	.00299	1.70	0	1
249	T.bub.	27.05.82	6.4	8.0	3.05	1	1	0.0129	0.1612	0.1795	2.24	.00135	0.75	1	2
250	T.bub.	27.05.82	5.7	5.9	3.19	1	2	0.0243	0.4119	0.1078	1.83	.00156	1.07	3	2
251	T.bub.	27.05.82	5.7	5.8	3.13	1	2	0.0167	0.2893	0.2000	3.45	.00095	0.48	2	2
252	T.bub.	27.05.82	8.0	13.8	2.69	1	2	0.0300	0.2174	0.2676	1.94	.00080	0.30	1	2
253	T.bub.	27.05.82	9.9	24.1	2.48	2	2	0.1258	0.5220	0.4434	1.84	.00127	0.28	1	2
254	T.bub.	27.05.82	2.0	0.2	2.50	0	-1	0	0	-1	-1	-1	-1	0	0
255	T.bub.	27.05.82	6.4	7.5	2.86	1	2	0.0228	0.3040	0.1433	1.91	.00163	0.81	3	2
256	T.bub.	27.05.82	6.2	6.7	2.81	1	2	-1	-1	0.1282	1.91	.00133	0.88	1	2
257	T.bub.	27.05.82	6.2	7.2	3.02	1	1	0.0056	0.0778	0.0962	1.34	.00125	1.65	1	2
258	T.bub.	27.05.82	6.5	7.4	2.69	1	2	0.0186	0.2513	0.1038	1.40	.00093	0.89	1	2
259	T.bub.	27.05.82	10.0	27.4	2.74	2	2	0.1502	0.5482	0.4430	1.61	.00109	0.23	2	1
260	T.bub.	27.05.82	12.0	47.7	2.76	2	2	0.2842	0.5958	0.7811	1.64	.00094	0.01	0	1
261	T.bub.	27.05.82	8.3	5.6	0.98	1	2	0.0945	1.6875	0.2221	3.95	.00085	0.38	1	2
262	T.bub.	27.05.82	6.9	9.5	2.89	1	1	0.0539	0.5674	0.2626	2.76	.00123	0.43	1	2
263	T.bub.	27.05.82	10.8	34.2	2.71	2	2	0.1969	0.5757	0.4345	1.27	.00061	0.14	1	3
264	T.bub.	27.05.82	6.9	10.2	3.10	1	2	0.0294	0.2882	0.1624	1.59	.00107	0.65	2	2

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Data sheet No. 10.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
265	T.bub.	27.05.82	7.0	8.7	2.54	1	2	0.0288	0.3310	0.1515	1.74	.00167	1.10	1	1
266	T.bub.	27.05.82	6.0	6.2	2.87	1	1	0.1270	1.4589	0.1162	1.87	.00106	0.91	1	1
267	T.bub.	27.05.82	6.5	7.8	2.84	1	1	0.0083	0.1064	0.1303	1.67	.00127	0.97	1	3
268	T.bub.	27.05.82	6.5	8.0	2.91	1	1	0.0028	0.0350	0.1574	1.97	-1	-1	2	3
269	T.bub.	27.05.82	3.3	0.9	2.50	0	2	0	0	0.0231	2.57	-1	-1	0	0
270	T.bub.	22.06.82	2.9	0.7	2.87	0	2	0	0	0.0330	4.71	-1	-1	0	0
271	T.bub.	22.06.82	3.5	1.2	2.80	0	2	0	0	0.0334	2.78	-1	-1	0	0
272	T.bub.	22.06.82	3.2	0.8	2.44	0	2	0	0	0.0414	5.18	-1	-1	1	0
273	T.bub.	22.06.82	3.7	1.4	2.76	0	2	-1	-1	0.0244	1.74	-1	-1	0	0
274	T.bub.	22.06.82	6.0	6.8	3.15	1	1	0.0020	0.0323	0.1476	2.17	-1	-1	1	0
275	T.bub.	22.06.82	8.5	16.4	2.67	1	2	0.0997	0.6079	0.3970	2.42	-1	-1	1	2
276	C.mus.	22.06.82	9.8	7.2	0.76	-1	-1	-1	-1	-1	-1	-1	-1	2	0
277	G.med.	22.06.82	10.5	9.3	0.80	-1	2	-1	-1	-1	-1	-1	-1	0	2
278	C.mus.	22.06.82	7.5	4.9	1.16	-1	1	-1	-1	-1	-1	-1	-1	2	3
279	L.pho.	22.06.82	9.1	10.1	1.34	-1	-1	-1	-1	-1	-1	-1	-1	2	0
281	T.bub.	22.06.82	9.6	9.2	1.04	2	2	0.0929	1.0079	0.4323	4.69	-1	-1	0	1
302	T.bub.	17.07.82	2.6	0.3	1.70	0	-1	0	0	0.0025	0.83	-1	-1	0	0

Site: PORTWRINKLE

Data sheet No. 11.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
303	T.bub.	21.07.82	3.0	0.5	1.85	0	-1	0	0	0.0083	1.66	-1	-1	0	3
304	T.bub.	28.07.82	7.5	9.8	2.32	1	2	0.0267	0.2724	0.1335	1.36	.00100	0.75	2	1
305	T.bub.	21.07.82	3.8	0.9	1.64	0	2	0.0065	0.7222	0.0328	3.64	-1	-1	2	3
306	T.bub.	21.07.82	8.4	12.7	2.14	2	2	0.0276	0.2173	0.1586	1.25	.00090	0.57	1	1
307	T.bub.	21.07.82	4.5	2.1	2.30	0	2	0.0045	0.2143	0.0309	1.47	-1	-1	2	3
308	T.bub.	21.07.82	4.3	1.8	2.26	0	-1	-1	-1	0.0242	1.34	-1	-1	2	2
309	T.bub.	21.07.82	3.3	0.8	2.23	0	-1	0	0	0.0117	1.46	-1	-1	1	2
320	T.bub.	10.08.82	4.5	2.8	3.07	0	2	0.0098	0.3500	0.0582	2.07	-1	-1	2	3
321	T.bub.	10.08.82	4.4	2.4	2.82	0	2	0	0	0.0766	3.19	-1	-1	3	1
322	T.bub.	10.08.82	3.4	1.8	4.58	0	-1	-1	-1	0.0380	2.11	-1	-1	1	3
323	T.bub.	10.08.82	5.0	3.2	2.56	0	2	0.0088	0.2750	0.0729	2.28	-1	-1	1	1
324	T.bub.	10.08.82	3.8	1.2	2.19	0	-1	-1	-1	0.0580	4.83	-1	-1	1	2
325	T.bub.	10.08.82	3.5	1.0	2.33	0	-1	-1	-1	0.0402	4.02	-1	-1	1	3
326	T.bub.	10.08.82	7.2	10.2	2.73	1	2	-1	-1	0.1904	1.87	-1	-1	2	2
329	T.bub.	18.08.82	8.5	14.7	2.39	1	1	0.0797	0.5422	0.2815	1.91	.00865	3.07	1	1
330	T.bub.	19.08.82	10.7	35.4	2.89	2	2	0.1465	0.4138	0.4599	1.30	.00261	0.57	1	0
331	T.bub.	19.08.82	7.6	12.5	2.85	1	2	0.0234	0.1872	0.1945	1.56	.00456	2.34	2	1

Site: PORTWRINKLE Data sheet No. 12

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
332	T.bub.	19.08.82	7.4	10.8	2.67	1	2	0.0267	0.2472	0.2070	1.92	-1	-1	1	1
333	T.bub.	19.08.82	5.0	3.2	2.56	0	-1	-1	-1	0.0558	1.74	-1	-1	2	1
334	T.bub.	19.08.82	6.8	8.6	2.74	1	2	-1	-1	0.2117	2.46	.00207	0.98	2	2
335	T.bub.	19.08.82	4.0	1.7	2.67	1	-1	-1	-1	0.0315	1.85	-1	-1	0	1
336	T.bub.	19.08.82	4.5	2.4	2.63	0	-1	-1	-1	0.0395	1.65	-1	-1	1	2
337	T.bub.	19.08.82	6.5	8.2	2.99	1	-1	-1	-1	0.1585	1.93	.00131	0.80	2	2
338	T.bub.	19.08.82	3.9	1.5	2.53	0	-1	-1	-1	0.0284	1.89	-1	-1	2	2
339	T.bub.	19.08.82	5.2	3.3	2.35	0	1	-1	-1	0.0871	2.64	-1	-1	1	3
340	T.bub.	19.08.82	4.6	2.4	2.47	0	2	0.0025	0.1042	0.0543	2.26	.00181	3.34	1	2
341	T.bub.	19.08.82	3.8	1.5	2.73	0	-1	-1	-1	0.0653	4.35	-1	-1	3	3
342	T.bub.	19.08.82	5.1	3.3	2.49	0	2	0.0087	0.2636	0.0692	2.10	.00034	0.40	2	2
343	T.bub.	19.08.82	3.9	1.3	2.19	0	1	-1	-1	0.0301	2.32	-1	-1	2	2
344	T.bub.	19.08.82	4.0	2.0	3.13	0	1	0	0	0.0616	3.08	-1	-1	2	2
345	T.bub.	19.08.82	5.2	3.6	2.56	0	2	-1	-1	0.1046	2.91	.0090	8.65	3	2
346	T.bub.	19.08.82	4.6	2.2	2.26	0	2	0.0059	0.2682	0.0404	1.84	-1	-1	1	3
347	T.bub.	20.08.82	4.0	1.7	2.66	0	1	0	0	0.0561	3.30	-1	-1	3	3
348	T.bub.	20.08.82	4.2	2.5	3.37	0	1	0	0	0.0805	3.22	-1	-1	3	3

Site: PORTWRINKLE Data sheet No. 13.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
349	T.bub.	20.08.82	5.0	3.1	2.48	0	1	0	0	0.0496	1.60	.00033	0.66	1	3
350	T.bub.	20.08.82	4.6	2.2	2.26	0	2	0.0050	0.2272	0.0625	2.84	.00059	0.94	3	3
351	T.bub.	20.08.82	4.6	2.2	2.26	0	1	-1	-1	0.0675	3.07	-1	-1	3	3
352	T.bub.	20.08.82	5.7	4.7	2.53	0	2	0.0126	0.2681	0.1582	3.37	-1	-1	3	3
353	T.bub.	20.08.82	3.6	1.0	2.14	0	-1	0	0	0.0358	3.58	-1	-1	0	2
355	T.bub.	20.08.82	9.8	28.6	3.04	2	2	0.1094	0.3825	0.4997	1.75	-1	-1	1	3
356	T.bub.	20.08.82	10.0	33.5	3.35	2	2	0.1091	0.3257	0.7910	2.36	-1	-1	1	2
357	T.bub.	20.08.82	7.9	13.7	2.78	1	2	0.0331	0.2416	0.3149	2.30	-1	-1	1	2
394	T.bub.	18.10.82	6.0	5.8	2.68	1	2	0.0203	0.3500	0.1524	2.63	.00302	1.98	3	1
395	T.bub.	18.10.82	4.6	2.8	2.88	0	2	0.0087	0.3107	0.0425	1.52	-1	-1	2	2
396	T.bub.	18.10.82	3.8	1.7	3.10	0	2	-1	-1	0.0204	1.20	-1	-1	2	0
397	T.bub.	18.10.82	8.0	13.3	2.60	2	1	0.0450	0.3383	0.2529	1.90	.00133	0.53	0	2
398	T.bub.	18.10.82	8.2	14.7	2.67	2	2	0.0740	0.4789	0.1830	1.24	.00143	0.57	1	2
399	T.bub.	18.10.82	10.5	28.1	2.43	2	2	0.1038	0.3694	0.5071	1.80	.00208	0.41	1	1
400	G.med.	18.10.82	4.2	0.6	0.81	-1	-1	-1	-1	-1	-1	-1	-1	0	0
401	C.mus.	18.10.82	7.1	4.8	1.34	-1	-1	-1	-1	-1	-1	-1	-1	2	0
403	T.bub.	18.10.82	5.4	4.6	2.92	1	1	0.0074	0.1609	0.0780	1.70	.00081	1.03	2	3

Site: PORTWRINKLE Data sheet No. 14.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
404	T.bub.	18.10.82	7.7	15.2	3.33	2	2	0.0554	0.3645	0.2564	1.69	.00198	0.46	0	3
405	T.bub.	18.10.82	7.8	8.4	1.77	2	1	0.0639	0.7607	0.2385	2.84	.00218	0.90	3	1
406	T.bub.	18.10.82	4.5	3.1	3.40	0	1	0	0	0.0259	0.84	-1	-1	3	1
407	T.bub.	18.10.82	4.7	3.1	2.99	0	2	0.0071	0.2290	0.0324	1.04	-1	-1	0	2
408	T.bub.	18.10.82	6.5	7.5	2.73	1	2	0.0207	0.276	0.1224	1.63	-1	-1	3	1
410	T.bub.	15.11.82	4.4	2.1	2.47	0	1	0	0	0.0481	2.29	-1	-1	2	2
411	T.bub.	15.11.82	4.4	2.0	2.35	0	1	0	0	0.0405	2.03	-1	-1	0	3
412	T.bub.	15.11.82	3.6	1.1	2.36	0	2	0.0007	0.0636	0.0205	1.86	-1	-1	1	2
413	T.bub.	15.11.82	9.2	23.6	3.03	2	2	0.1716	0.7271	0.4668	1.98	.00256	0.50	1	2
414	T.bub.	15.11.82	8.0	12.6	2.46	2	2	0.0925	0.7341	0.1408	1.14	-1	-1	2	3
415	T.bub.	15.11.82	5.0	2.7	2.16	0	1	0.0003	0.1111	0.0355	1.32	-1	-1	3	3
416	T.bub.	15.11.82	5.2	3.6	2.56	0	1	0.0012	0.0333	0.0896	2.49	-1	-1	3	3
417	T.bub.	15.11.82	4.9	3.0	2.55	0	2	0.0113	0.3767	0.0530	1.77	-1	-1	2	3
418	T.bub.	15.11.82	4.4	2.8	3.29	0	1	0	0	0.0645	2.30	-1	-1	2	2
419	T.bub.	15.11.82	5.7	6.2	3.35	0	1	0.0225	0.3630	0.0839	1.35	-1	-1	3	2
420	T.bub.	15.11.82	9.0	21.8	2.99	2	2	0.2297	0.0537	0.3584	1.64	.00603	1.68	2	1
421	T.bub.	15.11.82	4.2	3.6	4.86	0	1	0.0014	0.0389	0.0916	2.54	-1	-1	3	1

Site: PORTWRINKLE

Data sheet No. 15.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
422	T.bub.	15.11.82	4.9	3.6	3.06	0	1	0.0031	0.0861	0.0698	1.94	.00033	0.46	1	3
423	T.bub.	15.11.82	4.0	1.5	2.35	0	2	0.0052	0.3467	0.0384	2.56	-1	-1	2	3
425	T.bub.	15.11.82	5.5	4.2	2.52	0	1	0.0024	0.0571	0.1172	2.79	.00134	1.14	1	3
426	T.bub.	15.11.82	5.2	4.4	3.13	1	1	0.0298	0.6772	0.1017	2.31	.00047	0.46	2	3
427	T.bub.	15.11.82	4.5	2.0	2.19	0	2	0.0036	0.1800	0.0352	1.76	-1	-1	0	3
428	T.bub.	15.11.82	6.0	5.0	2.31	1	1	0.0146	0.2920	0.1351	2.70	.00121	0.90	2	3
429	T.bub.	15.11.82	3.5	1.0	2.33	0	-1	0	0	0.0335	3.35	-1	-1	3	3
430	T.bub.	15.11.82	5.7	4.8	2.59	1	2	0.0286	0.5583	0.0939	1.95	-1	-1	3	1
431	T.bub.	15.11.82	5.0	3.3	2.64	0	1	0	0	0.0564	1.71	-1	-1	2	2
432	T.bub.	14.02.83	4.3	2.4	3.02	0	-1	0	0	0.0706	2.94	.00016	0.23	3	2
433	T.bub.	14.02.83	5.5	4.1	2.46	0	2	0.0230	0.5609	0.0693	1.69	.00017	0.24	2	1
434	T.bub.	14.02.83	4.7	2.9	2.79	0	2	0.0140	0.4828	0.0901	3.11	.00040	0.44	2	2
435	T.bub.	14.02.83	3.8	1.5	2.73	0	1	0	0	0.0744	4.96	.00056	0.76	2	3
436	T.bub.	14.02.83	5.6	4.9	2.79	0	2	0.0252	0.5143	0.1141	2.32	.00021	0.18	2	2
437	T.bub.	14.02.83	4.3	1.9	2.39	0	1	0	0	0.0361	1.9	-1	-1	2	1
438	T.bub.	28.02.83	5.9	4.7	2.29	0	2	0.0184	0.3915	0.0866	1.84	.00001	0.02	2	2
439	T.bub.	28.02.83	7.8	12.1	2.55	1	1	0.3283	2.7132	0.1444	1.19	.00002	0.02	2	2

Site: PORTWRINKLE

Data sheet No. 16.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
440	T.bub.	28.02.83	6.7	8.5	2.83	1	2	0.4402	5.1789	0.3121	3.67	.00006	0.02	0	2
441	T.bub.	28.02.83	8.5	7.2	1.17	1	2	0.3103	4.3097	0.3260	4.53	.00003	0.01	2	3
442	T.bub.	28.02.83	10.5	30.0	2.59	2	2	1.0946	3.6487	0.5996	1.99	.00003	0.00	2	2
443	T.bub.	02.03.83	5.2	4.3	3.06	0	1	0	0	0.0653	1.52	.00001	0.01	2	2
444	T.bub.	02.03.83	6.2	6.0	2.52	1	1	0.0528	0.8800	0.1014	1.69	-1	-1	1	2
445	T.bub.	02.03.83	6.2	5.0	2.10	0	2	0.0201	0.402	0.1141	2.28	.00001	0.01	2	1
446	T.bub.	02.03.83	5.1	3.4	2.56	0	1	0	0	0.0737	2.17	.00002	0.00	2	2
447	T.bub.	02.03.83	5.0	3.0	2.40	0	1	0	0	0.0580	1.93	-1	-1	2	3
448	T.bub.	02.03.83	12.0	45.0	2.60	2	2	3.5702	7.9338	2.0844	4.63	.0015	0.07	1	1
449	T.bub.	25.04.83	6.6	8.1	2.82	1	2	0.0528	0.6518	0.1811	2.24	.00019	0.10	2	1
450	T.bub.	25.04.83	7.9	12.8	2.59	2	1	0.0408	0.3187	0.2687	2.10	.00041	0.15	2	1
451	T.bub.	25.04.83	7.1	10.0	2.79	1	2	0.0622	0.6220	0.2116	2.17	-1	-1	1	3
452	T.bub.	25.04.83	8.8	19.3	2.83	2	2	0.0690	0.3575	0.4116	2.13	.00064	0.16	1	1
453	T.bub.	25.04.83	5.4	4.0	2.54	1	1	0.0041	0.1025	0.0688	1.72	.00014	0.20	1	3
454	T.bub.	25.04.83	6.3	7.8	3.12	1	1	0.0020	0.0256	0.2118	2.71	.00020	0.09	2	3
455	T.bub.	26.04.83	6.4	6.8	2.59	1	1	0.0035	0.0515	0.1585	2.33	-1	-1	0	3
456	T.bub.	26.06.83	6.5	8.6	3.13	1	1	0.0084	0.0976	0.1306	1.52	.00012	0.09	1	2

Site: PORTWRINKLE

Data sheet No.17.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met.	Mic.
457	T.bub.	26.04.83	8.9	18.8	2.66	2	2	0.0867	0.4612	0.3574	1.90	-1	-1	1	1
458	T.bub.	26.04.83	6.7	7.8	2.59	1	1	0.0988	1.2666	0.1561	2.00	.00017	0.12	1	1
459	T.bub.	26.04.83	9.0	18.7	2.56	2	2	0.1683	0.9000	0.4123	2.20	.00049	1.19	2	1
460	T.bub.	27.04.83	7.3	7.2	1.85	2	2	0.0371	0.5153	0.1913	2.66	.00018	0.09	1	3
461	T.bub.	27.04.83	5.8	5.0	2.56	1	2	0.0176	0.3520	0.1114	2.23	.00018	0.16	2	3
462	T.bub.	27.04.83	8.3	12.6	2.20	2	1	0.0701	0.5563	0.1396	1.10	.00029	0.21	2	2
463	T.bub.	27.04.83	7.7	12.0	2.63	1	1	0.0559	0.4658	0.1627	1.36	.00015	0.09	2	2
464	T.bub.	27.04.83	6.7	8.1	2.69	1	2	0.0271	0.3346	0.1520	1.88	.00022	0.15	2	2

Site: ABERYSTWYTH Data sheet No. 1.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
153	T.bub.	10.12.81	8.0	15.7	3.06	1	1	0.0400	0.2548	0.1400	4.58	-1	-1	0	0
154	T.bub.	10.12.81	6.0	5.7	2.60	-1	2	0.0300	0.5263	0.0750	1.32	-1	-1	0	1
155	T.bub.	10.12.81	10.5	29.5	2.55	2	2	0.0700	0.2373	0.5000	1.69	-1	-1	0	1
156	T.bub.	10.12.81	4.6	4.2	4.30	0	2	0.0200	0.4762	0.1200	2.86	-1	-1	0	0
157	T.bub.	10.12.81	8.0	15.0	2.90	1	1	0.2600	1.7333	0.2600	6.19	-1	-1	1	1
158	T.bub.	10.12.81	9.0	25.7	3.50	1	2	0.5800	2.2568	1.1200	4.36	-1	-1	0	1
159	T.bub.	10.12.81	6.5	9.9	3.60	1	1	0.1200	1.2121	0.1600	1.62	-1	-1	0	0
160	T.bub.	10.12.81	9.5	24.9	2.89	1	2	0.0300	0.1205	0.1800	0.72	-1	-1	0	0
161	T.bub.	10.12.81	5.2	4.8	3.40	0	2	0.0200	0.4167	0.1850	3.85	-1	-1	0	0
162	T.bub.	10.12.81	5.6	7.7	4.30	0	1	0	0	0.2800	3.67	-1	-1	0	0
163	T.bub.	10.12.81	4.5	3.3	3.60	0	1	0	0	0.0300	0.91	-1	-1	0	0
164	T.bub.	10.12.81	5.2	5.0	3.50	0	2	0	0	-1	-1	-1	-1	0	0
165	T.bub.	10.12.81	5.2	4.5	3.20	0	1	-1	-1	0.1800	4.00	-1	-1	0	0
170	T.bub.	10.12.81	4.6	4.1	4.20	0	1	-1	-1	0.0900	2.19	-1	-1	0	0
311	T.bub.	04.08.82	8.5	14.8	2.41	1	2	0.0341	0.2304	0.2259	1.53	.00230	1.02	0	0
312	T.bub.	04.08.82	9.0	22.8	3.13	1	2	0.0397	0.1741	0.3950	0.17	.00570	1.44	0	0
313	T.bub.	04.08.82	6.8	9.8	3.12	1	1	0.0099	0.1010	0.2128	2.17	.00190	0.89	2	0

Site: ABERYSTWYTH

Data sheet No. 2.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met.	Mic.
314	T.bub.	04.08.82	10.4	32.9	2.92	2	2	0.0755	0.2295	0.6147	1.87	-1	-1	0	0
315	T.bub.	04.08.82	7.7	13.5	2.96	1	2	0.0935	0.6926	0.1511	1.12	.00080	0.12	0	0
316	T.bub.	04.08.82	7.3	12.2	3.14	1	2	0.0152	0.1246	0.1673	1.37	.00182	1.87	0	0
317	T.bub.	04.08.82	7.2	11.1	2.97	1	1	0.0020	0.0180	0.2943	2.65	-1	-1	0	0
318	C.mus.	04.08.82	11.5	11.6	0.16	-1	-1	-1	-1	-1	-1	-1	-1	0	0
319	T.bub.	04.08.82	4.0	2.1	3.28	0	-1	-1	-1	0.0268	1.28	-1	-1	0	0
327	T.bub.	04.08.82	3.8	1.3	2.37	0	1	0	0	0.0449	3.45	-1	-1	0	0

Site: ROSCOFF

Data sheet No. 1.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
365	T.bub.	02.09.82	8.2	13.9	2.52	1	2	0.0526	0.378	0.2980	2.14	.00608	2.01	0	2
366	T.bub.	02.09.82	10.7	33.6	2.74	1	2	0.1243	0.370	0.8573	2.55	.01062	1.89	1	2
367	G.nig.	02.09.82	8.8	13.1	1.92	-1	-1	-1	-1	-1	-1	-1	-1	0	0
368	G.nig.	02.09.82	7.8	10.6	2.33	-1	-1	-1	-1	-1	-1	-1	-1	0	0
369	C.lyr.	02.09.82	5.2	1.9	1.35	-1	-1	-1	-1	-1	-1	-1	-1	0	0
370	C.mel.	02.09.82	5.1	2.7	2.03	-1	-1	-1	-1	-1	-1	-1	-1	0	0
371	T.bub.	02.09.82	5.7	4.4	2.38	0	-1	0	0	0.1380	3.14	.00047	0.34	2	2
372	T.bub.	02.09.82	4.8	2.7	2.44	0	1	0	0	0.0426	1.58	.00460	10.9	0	0
373	C.mel.	05.09.82	7.0	6.1	1.78	-1	-1	-1	-1	-1	-1	-1	-1	0	0
374	G.med.	05.09.82	6.2	2.2	0.92	-1	-1	-1	-1	-1	-1	-1	-1	0	2
375	G.med.	05.09.82	7.0	3.0	0.87	-1	-1	-1	-1	-1	-1	-1	-1	0	1
376	T.bub.	02.09.82	8.7	19.9	3.02	1	1	0.0662	0.333	0.2850	1.43	.00068	0.24	0	1
377	T.bub.	02.09.82	9.8	24.2	2.57	1	2	0.1137	0.470	0.3100	1.28	.00053	0.17	0	1
378	T.bub.	02.09.82	5.2	4.2	2.99	0	1	0	0	0.1004	2.39	.03239	32.26	0	1
379	T.bub.	02.09.82	9.9	24.3	2.50	1	2	-1	-1	0.4349	1.79	.00032	0.07	1	1
380	T.bub.	02.09.82	5.5	5.0	3.01	0	1	0	0	0.1172	2.34	.02497	21.31	0	2
381	T.bub.	02.09.82	11.5	40.2	2.64	1	2	0.2119	0.527	1.2600	3.13	.01662	1.32	0	1

Site: ROSCOFF

Data sheet No. 2.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met.	Mic.
382	T.bub.	02.09.82	14.3	74.9	2.56	2	2	-1	-1	1.3909	1.85	.01342	0.97	0	0
383	T.bub.	02.09.82	5.4	4.4	2.79	0	2	-1	-1	-1	-1	-1	-1	1	0
384	T.bub.	02.09.82	8.3	13.6	2.38	1	2	-1	-1	0.1841	1.35	.01327	7.21	1	0
385	T.bub.	02.09.82	8.5	5.8	0.94	1	1	-1	-1	-1	-1	-1	-1	0	3
386	T.bub.	02.09.82	9.8	22.9	2.43	1	2	0.1086	0.474	0.5970	2.61	.02154	3.61	2	2
387	T.bub.	02.09.82	5.9	4.9	2.39	0	2	0.0311	0.635	0.0700	1.43	.00487	6.96	0	0
388	T.bub.	02.09.82	5.3	4.1	2.75	0	1	0	0	0.0702	1.71	.00025	0.36	0	0
389	T.bub.	02.09.82	5.7	4.6	2.48	0	2	-1	-1	0.0765	1.66	.00272	3.55	0	0
391	T.bub.	02.09.82	6.7	8.3	2.76	1	2	0.0381	0.459	0.2181	2.63	.04324	19.83	1	1
392	T.bub.	02.09.82	4.8	3.6	3.25	0	1	0	0	0.1025	2.85	-1	-1	0	0
393	T.bub.	02.09.82	8.2	5.9	1.07	1	1	0.0464	0.786	0.3544	6.01	.02419	6.83	1	0

Site: WEMBURY

Data sheet No. 1.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
26	C.mus.	07.02.81	13.0	12.1	0.55	-1	-1	-1	-1	-1	-1	-1	-1	0	0
27	C.mus.	07.02.81	9.8	5.4	0.57	-1	-1	-1	-1	-1	-1	-1	-1	0	0
28	C.mel.	07.02.81	8.7	8.7	1.32	-1	-1	-1	-1	-1	-1	-1	-1	0	0
29	T.bub.	07.02.81	7.8	8.1	1.71	-1	2	-1	-1	-1	-1	-1	-1	1	1
36	T.bub.	19.05.81	9.5	14.0	1.63	-1	-1	-1	-1	-1	-1	-1	-1	0	1
38	T.bub.	19.05.81	9.0	12.4	1.70	-1	1	-1	-1	-1	-1	-1	-1	2	1
39	T.bub.	19.05.81	10.0	18.7	1.87	-1	1	-1	-1	-1	-1	-1	-1	0	1
40	G.fla.	18.05.81	4.0	0.7	1.09	-1	-1	-1	-1	-1	-1	-1	-1	0	0
62	T.bub.	06.06.81	7.2	11.6	3.10	1	-1	-1	-1	-1	-1	-1	-1	3	2
63	T.bub.	06.06.81	9.0	9.9	1.35	2	2	-1	-1	-1	-1	-1	-1	0	3
64	T.bub.	06.06.81	6.5	8.3	3.02	1	-1	-1	-1	-1	-1	-1	-1	1	1
65	T.bub.	06.06.81	9.5	20.7	2.41	2	-1	-1	-1	-1	-1	-1	-1	3	1
66	G.nig.	04.06.81	9.0	14.1	1.93	-1	-1	-1	-1	-1	-1	-1	-1	0	0
67	T.bub.	06.06.81	6.8	6.7	2.13	1	-1	-1	-1	-1	-1	-1	-1	2	1
72	T.bub.	06.06.81	1.7	0.04	0.81	0	-1	-1	-1	-1	-1	-1	-1	0	0
75	T.bub.	15.07.81	2.0	0.2	1.88	0	-1	-1	-1	-1	-1	-1	-1	2	3
86	C.mel.	15.07.81	9.0	17.5	2.40	-1	-1	-1	-1	-1	-1	-1	-1	0	0

Site: WEMBURY

Data sheet No. 2.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
87	G.med.	15.07.81	18.5	51.5	0.81	-1	2	-1	-1	-1	-1	-1	-1	1	0
88	T.bub.	15.07.81	4.5	2.1	2.30	0	-1	-1	-1	-1	-1	-1	-1	2	2
89	T.bub.	15.07.81	4.5	2.7	2.96	0	-1	-1	-1	-1	-1	-1	-1	3	3
90	C.mus	15.07.81	4.3	1.4	1.76	-1	-1	-1	-1	-1	-1	-1	-1	0	1
91	C.mel.	03.08.81	5.5	2.8	1.69	-1	-1	-1	-1	-1	-1	-1	-1	0	3
138	T.bub.	12.11.81	7.0	8.1	2.36	1	2	0.0400	0.4900	0.2000	2.47	-1	-1	1	2
139	T.bub.	12.11.81	6.0	5.6	2.59	1	1	0.0250	0.4460	0.1000	1.78	-1	-1	2	3
140	G.med.	12.11.81	11.0	12.0	0.90	-1	-1	-1	-1	-1	-1	-1	-1	0	2
141	T.bub.	12.11.81	5.0	4.8	3.84	0	1	0.0600	1.2500	0.0900	1.87	-1	-1	1	3
142	C.mus.	12.11.81	9.0	6.0	0.82	-1	-1	-1	-1	-1	-1	-1	-1	0	0
143	T.bub.	12.11.81	6.5	8.4	3.06	1	1	0.0600	0.7100	0.2000	2.38	-1	-1	3	3
144	T.bub.	12.11.81	6.8	7.2	2.29	1	2	0.0400	0.5500	0.2100	2.91	-1	-1	1	2
145	C.mel.	12.11.81	6.5	5.8	2.11	-1	2	-1	-1	-1	-1	-1	-1	0	1
147	T.bub.	12.11.81	5.5	4.4	2.64	0	1	0.0300	0.6800	0.0800	1.80	-1	-1	0	2
148	T.bub.	12.11.81	10.0	31.0	3.10	2	2	0.7100	2.2900	0.2950	0.95	-1	-1	2	0
221	T.bub.	10.03.82	7.0	8.9	2.59	1	2	0.0297	0.3340	0.1277	1.43	-1	-1	2	1
222	C.mus.	10.03.82	12.0	24.0	1.39	-1	2	-1	-1	0.4812	2.00	-1	-1	2	1

Site: WEMBURY

Data sheet No. 3.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
223	T.bub.	10.03.82	5.0	3.9	3.12	0	2	0.1690	4.3330	0.1100	2.82	-1	-1	0	3
224	C.mus.	10.03.82	13.0	18.5	0.84	-1	1	-1	-1	-1	-1	-1	-1	0	0
225	T.bub.	10.03.82	7.0	8.4	2.45	1	2	0.2770	3.2970	0.1668	1.98	-1	-1	1	3
226	T.bub.	10.03.82	4.5	2.8	3.07	0	2	0	0	0.8910	3.18	-1	-1	2	3
227	T.bub.	10.03.82	4.4	2.1	2.47	0	1	0	0	0.0334	1.59	-1	-1	2	2
228	T.bub.	10.03.82	5.5	4.7	2.83	0	2	0.0178	0.3780	0.0945	2.00	-1	-1	1	3
282	T.bub.	05.07.82	8.0	5.2	2.97	1	2	0.0613	0.4033	0.2250	1.48	-1	-1	1	2
282	T.bub.	05.07.82	3.7	1.7	3.36	0	-1	0	0	0.0362	1.08	-1	-1	1	0
284	T.bub.	05.07.82	3.6	1.2	2.57	0	-1	0	0	0.0307	2.56	-1	-1	1	0
285	T.bub.	05.07.82	2.4	0.4	2.89	0	-1	0	0	0.0082	2.05	-1	-1	1	0
286	T.bub.	05.07.82	7.2	11.2	3.00	1	1	0.0093	0.0830	0.2511	2.24	-1	-1	2	2
287	T.bub.	05.07.82	3.1	0.7	2.35	0	2	0.0032	0.4571	0.0070	1.00	-1	-1	0	0
288	T.bub.	05.07.82	3.8	1.1	2.00	0	2	0	0	0.0228	2.07	-1	-1	1	0
289	T.bub.	05.07.82	7.6	11.7	2.66	1	1	0.0043	0.0368	0.1708	1.46	-1	-1	2	1
290	T.bub.	05.07.82	3.0	0.7	2.59	0	-1	0	0	0.0108	1.54	-1	-1	0	1
300	T.bub.	05.07.82	4.0	1.6	2.50	0	2	0.0002	0.0125	0.0590	3.69	-1	-1	1	2
301	T.bub.	05.07.82	3.2	0.9	2.75	0	2	0	0	0.0098	1.09	-1	-1	0	3
328	T.bub.	16.08.82	14.3	91.8	-1	3	2	0.5220	0	1.3421	1.46	.01232	0.92	0	0

Site: WIDEMOUTH

Data sheet No. 1.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met	Mic
1	T.bub.	21.11.80	7.0	5.0	1.46	1	-1	-1	-1	-1	-1	-1	-1	1	3
2	T.bub.	21.11.80	8.0	9.0	1.76	1	-1	-1	-1	-1	-1	-1	-1	1	3
3	T.bub.	21.11.80	6.5	3.5	1.28	1	-1	-1	-1	-1	-1	-1	-1	1	2
55	C.mus.	01.06.81	12.0	14.9	0.86	-1	2	-1	-1	0.2	1.34	-1	-1	0	1
56	C.mus.	01.06.81	8.2	5.5	0.99	-1	1	-1	-1	-1	-1	-1	-1	0	2
57	C.mus.	01.06.81	8.0	5.0	0.98	-1	-1	-1	-1	-1	-1	-1	-1	0	2
58	T.bub.	01.06.81	6.7	4.0	1.33	1	-1	-1	-1	0.06	1.5	-1	-1	-1	2
59	T.bub.	01.06.81	7.0	4.6	1.34	3	2	-1	-1	0.045	0.98	-1	-1	-1	1
60	T.bub.	01.06.81	15.5	60.6	2.76	1	2	-1	-1	1.03	1.70	-1	-1	1	1
137	T.bub.	28.10.81	7.0	10.9	3.18	1	2	0.04	0.3670	-1	-1	-1	-1	0	1
469	T.bub.	12.06.83	2.1	0.23	2.45	0	-1	-1	-1	0.0064	2.78	-1	-1	0	0
470	T.bub.	12.06.83	2.4	0.39	2.82	0	-1	-1	-1	0.0080	2.05	-1	-1	0	0
471	G.med.	12.06.83	8.1	3.9	0.73	-1	-1	-1	-1	-1	-1	-1	-1	0	1
472	T.bub.	12.06.83	2.6	0.43	2.45	0	-1	-1	-1	0.0090	2.09	-1	-1	0	0
473	P.pol.	12.06.83	4.3	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	0	0
474	G.nig.	12.06.83	4.6	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	0	0
475	T.bub.	12.06.83	6.4	7.9	3.01	1	1	0.0170	0.2152	0.1563	1.98	.00042	0.27	0	0

Site: WIDEMOUTH

Data sheet No. 2.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met.	Mic.
476	T.bub.	12.06.83	10.0	27.4	2.74	2	2	0.1540	0.5620	0.3706	1.35	.00219	0.59	0	1
477	T.bub.	12.06.83	6.4	6.4	2.44	1	-1	-1	-1	0.0937	1.46	.00013	0.14	0	0
478	T.bub.	12.06.83	6.2	7.2	3.02	1	1	0.0055	0.0763	0.0961	1.50	.00030	0.31	1	2
479	T.bub.	12.06.83	6.5	7.2	2.62	1	2	0.0380	0.5277	0.1359	1.89	.00062	0.45	0	1
480	T.bub.	12.06.83	7.0	9.7	2.83	1	2	0.0340	0.3505	0.1417	1.46	.00055	0.38	0	3

Site: ST.JOHNS Data sheet No. 1.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met.	Mic.
45	C.lab.	21.05.81	5.0	1.4	1.1	-1	-1	-1	-1	-1	-1	-1	-1	0	2
46	C.lab.	21.05.81	5.0	1.5	1.2	-1	-1	-1	-1	-1	-1	-1	-1	0	0
47	C.lab.	21.05.81	5.1	1.7	1.3	-1	-1	-1	-1	-1	-1	-1	-1	0	1
48	C.lab.	21.05.81	5.2	1.5	1.1	-1	-1	-1	-1	-1	-1	-1	-1	0	2
49	C.lab.	21.05.81	5.1	1.6	1.2	-1	-1	-1	-1	-1	-1	-1	-1	0	2
50	C.lab.	21.05.81	5.2	1.9	1.9	-1	-1	-1	-1	-1	-1	-1	-1	0	0
51	C.lab.	21.05.81	5.4	1.8	1.1	-1	-1	-1	-1	-1	-1	-1	-1	0	1
52	C.lab.	21.05.81	4.9	1.6	1.4	-1	-1	-1	-1	-1	-1	-1	-1	0	0
53	C.lab.	21.05.81	4.2	1.2	1.6	-1	-1	-1	-1	-1	-1	-1	-1	0	1
54	C.lab.	21.05.81	4.9	5.5	1.3	-1	-1	-1	-1	-1	-1	-1	-1	0	1

Site: RINGMORE Data sheet No.1.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Liver % Liver	Infection	
														Met.	Mic.
92	L.pho.	13.08.81	4.5	1.3	1.43	-1	2	-1	-1	-1	-1	-1	-1	2	0
93	C.mus.	13.08.81	7.2	3.5	0.94	-1	-1	-1	-1	-1	-1	-1	-1	0	0
94	T.bub.	13.08.81	4.5	2.2	2.41	0	0	-1	-1	-1	-1	-1	-1	0	0
95	T.bub.	13.08.81	4.3	2.1	2.64	0	-1	-1	-1	-1	-1	-1	-1	0	1
96	T.bub.	13.08.81	5.5	4.6	2.76	1	1	-1	-1	-1	-1	-1	-1	3	3
97	T.bub.	13.08.81	4.2	2.2	2.97	0	0	-1	-1	-1	-1	-1	-1	0	2
99	G.med.	14.08.81	10.2	8.0	0.75	-1	1	-1	-1	-1	-1	-1	-1	0	2

Site: PRAWL POINT Data sheet No.1.

98	C.gal.	14.08.81	6.3	1.8	0.72	-1	2	-1	-1	-1	-1	-1	-1	0	0
100	L. pho.	14.08.81	8.5	8.5	1.38	-1	2	-1	-1	-1	-1	-1	-1	1	0
101	L. pho.	14.08.81	11.0	19.2	1.45	-1	2	-1	-1	-1	-1	-1	-1	3	0

Site: RINZY HEAD

Data sheet No. 1.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection Met.	Infection Mic.
21	L.pho.	27.01.81	6.0	4.0	1.95	-1	-1	-1	-1	-1	-1	-1	-1	0	0
22	L.pho.	27.01.81	5.0	3.5	2.8	-1	-1	-1	-1	-1	-1	-1	-1	0	0
23	L.pho.	27.01.81	7.5	5.5	1.30	-1	1	-1	-1	-1	-1	-1	-1	0	0
24	L.pho.	27.01.81	10.4	13.0	1.16	-1	2	-1	-1	-1	-1	-1	-1	0	0
25	L.pho.	27.01.81	6.5	4.0	1.46	-1	1	-1	-1	-1	-1	-1	-1	0	0
30	L.pho.	24.02.81	5.0	2.9	2.32	-1	2	-1	-1	-1	-1	-1	-1	0	0
31	L.pho.	24.02.81	4.0	1.7	2.66	-1	1	-1	-1	-1	-1	-1	-1	0	0
38	C.gal.	24.02.81	4.8	1.8	1.63	-1	-1	-1	-1	-1	-1	-1	-1	0	0

Site: NEWQUAY

Data sheet No. 1.

376	T.bub.	06.05.81	8.5	8.7	1.42	2	2	-1	-1	-1	-1	-1	-1	2	3

Site: SOUTH WALES

Data sheet No.1.

Case No.	Species	Date	Length cm	Weight grm	C.F. X 100	Age	Sex	Gonad weight grm	Gonad % body	Liver weight grm	Liver % body	Total Lipid of Liver grm	Lipid % Liver	Infection	
														Met.	Mic.
37	L. pho.	31.03.81	8.5	5.9	0.96	-1	-1	-1	-1	-1	-1	-1	-1	0	0
113	T. bub.	21.09.81	3.0	1.0	3.70	-1	0	-1	-1	-1	-1	-1	-1	0	0
114	G. med.	22.09.81	17.0	51.5	1.05	-1	-1	-1	-1	-1	-1	-1	-1	1	0
117	G. med.	21.09.81	12.5	16.3	0.83	-1	2	-1	-1	-1	-1	-1	-1	0	0

A P P E N D I X 11

a) CHI-SQUARE ANALYSIS OF HOST VARIABLES (X-AXIS) WITH MICROGEMMA INFECTION LEVELS (Y-AXIS)

i) Microgemma infection/length

Observed frequencies

11	2	2	9	24
10	10	18	24	62
16	26	24	22	88
22	27	13	5	67
59	65	57	60	241

Expected frequencies

6.0	6.5	5.7	5.9
15.4	16.7	14.7	15.2
21.5	23.7	20.8	21.9
16.7	18.1	15.8	16.4

Contributions to Chi-square

4.2	3.1	2.4	1.7
1.9	2.7	0.8	5.1
1.4	0.2	0.5	0.0
1.7	4.4	0.5	7.9

Chi-square = 38.23 on 9 df.

P = 0.00002

Therefore H_1 applies

ii) Microgemma infection/weight

Observed frequencies

11	1	3	9	24
7	12	19	24	62
15	26	24	23	88
21	28	12	6	67
54	67	58	62	241

Expected frequencies

5.4	6.7	5.8	6.2
13.9	17.2	14.9	16.0
19.7	24.5	21.2	22.6
15.0	18.6	16.1	17.6

Contributions to Chi-square

5.9	4.8	1.3	1.3
3.4	1.6	1.1	4.1
1.1	0.1	0.4	0.0
2.4	4.7	1.1	7.3

Chi-square = 40.61 on 9 df.

P = 0.000006

Therefore H_1 applies

iii) Microgemma infection/age

Observed frequencies

13	6	5	24
22	26	14	62
41	36	11	88
45	17	5	67
121	85	35	241

Expected frequencies

12.0	8.5	3.5
31.1	21.9	9.0
44.2	31.0	12.7
33.6	23.6	9.73

Contributions to Ch-square

0.1	0.7	0.6
2.7	0.8	2.8
0.2	0.8	0.2
3.9	1.8	2.3

Chi-square = 16.94 on 6 df.

P = 0.01

Therefore H_1 applies

iv) Microgemma infection with sex

Observed frequencies

4	15	19
21	33	54
29	44	73
32	25	57
86	117	203

Expected frequencies

8.0	11.0
22.9	31.1
30.9	42.1
24.1	32.9

Contributions to Chi-square

2.0	1.5
0.2	0.1
0.1	0.1
2.6	1.9

Chi-square = 8.44 on 3 df.

P = 0.04

Therefore H_1 applies

v) Microgemma infection with gonad weight

Observed frequencies

9	1	3	2	15
10	4	23	4	41
20	11	32	2	65
20	19	16	1	56
59	35	74	9	177

Expected frequencies

5.0	3.0	6.3	0.8
13.7	8.1	17.1	2.1
21.7	12.9	27.2	3.3
18.7	11.1	23.4	3.0

Contributions to Chi-square

3.2	1.3	1.8	1.8
1.0	2.1	2.0	1.7
0.1	0.3	0.8	0.5
0.1	5.7	2.3	1.3

Chi-square = 26.05 on 9 df.

P = 0.005

Therefore H_1 applies

vi) Microgemma infection with gonad weight as % body weight

Observed frequencies

8	1	2	3	14
10	7	12	13	42
20	12	19	14	65
19	15	12	10	56
57	35	45	40	177

Expected frequencies

4.5	2.8	3.6	3.2
13.5	8.3	10.7	9.5
20.9	12.9	16.5	14.7
18.0	11.1	14.2	12.7

Contributions to Chi-square

2.7	1.1	0.7	0.0
0.9	0.2	0.2	1.3
0.0	0.1	0.4	0.0
0.1	1.4	0.4	0.6

Chi-square = 9.94 on 9 df.

P > 0.05

Therefore H_o applies

vii) Microgemma infection with liver weight

Observed frequencies

8	1	1	6	16
4	7	15	16	42
14	20	20	23	77
17	17	15	12	61
43	45	51	57	196

Expected frequencies

5.5	3.6	4.2	4.6
9.2	9.6	10.9	12.2
16.9	17.7	20.0	22.3
13.4	14.0	15.9	17.7

Contributions to Chi-square

5.8	1.9	2.4	0.4
2.9	0.7	1.5	1.2
0.5	0.3	0.0	0.0
1.0	0.6	0.0	1.8

Chi-square = 28.5 on 9 df.

$P < 0.001$

Therefore H_1 applies

viii) Microgemma infection with liver weight % body weight

Observed frequencies

5	1	5	5	16
10	13	14	7	44
22	22	19	13	76
12	19	13	16	60
49	55	51	41	196

Expected frequencies

4.0	4.5	4.2	3.3
11.0	12.3	11.4	9.2
19.0	21.3	19.8	15.9
15.0	16.8	15.6	12.6

Contributions to Chi-square

0.3	2.7	0.2	0.8
0.1	0.0	0.6	0.5
0.5	0.0	0.0	0.5
0.6	0.3	0.4	0.9

Chi-square = 8.485 on 9 df.

$P > 0.05$

Therefore H_o applies

ix) Microgemma infection with condition factor

Observed frequencies

3	5	5	11	24
16	22	15	9	62
19	21	26	22	88
11	25	11	20	67
49	73	57	62	241

Expected frequencies

5.0	7.3	5.7	6.0
12.6	18.8	14.7	16.0
18.3	26.7	20.8	22.3
13.9	20.3	15.8	17.0

Contributions to Chi-square

0.8	0.7	0.1	4.1
0.9	0.5	0.0	3.0
0.0	1.2	1.3	0.0
0.6	1.1	1.5	0.5

Chi-square = 16.3 on 9 df.

$P > 0.05$

Therefore H_o applies

x) *Microgemma* infection with total lipid weight of liver

Observed frequencies

0	1	0	2	3
8	10	3	3	24
19	15	0	1	35
13	3	5	1	22
40	29	8	7	84

Expected frequencies

1.4	1.0	0.3	0.3
11.4	8.3	2.3	2.0
16.7	12.1	3.3	2.9
10.5	7.6	2.1	1.8

Contributions to Chi-square

1.4	0.0	0.3	12.3
1.0	0.4	0.2	0.5
0.3	0.7	3.3	1.3
0.6	2.8	4.0	0.4

Chi-square - 29.49 on 9 df.

P = 0.0005

Therefore H_1 applies

xi) *Microgemma* infection with total lipid percentage of liver

Observed frequencies

0	1	2	0	3
12	2	5	6	24
12	7	10	4	35
6	5	6	6	22
30	15	23	16	84

Expected frequencies

1.1	0.5	0.8	0.6
8.9	4.5	6.8	4.8
11.8	5.9	9.0	6.3
8.2	4.1	6.3	4.4

Contributions to Chi-square

1.1	0.4	1.7	0.6
1.1	1.4	0.5	0.3
0.0	0.2	0.1	0.8
0.6	0.2	0.0	0.6

Chi-square = 9.536 on 9 df.

P > 0.05

Therefore H_o applies

xii) *Microgemma* infection with condition factor

Observed frequencies

3	5	5	11	24
16	22	15	9	62
19	21	26	22	88
11	25	11	20	67
49	73	57	62	241

Expected frequencies

5.0	7.3	5.7	6.0
12.6	18.8	14.7	16.0
18.3	26.7	20.8	22.3
13.9	20.3	15.8	17.0

Contributions to Chi-square

0.8	0.7	0.1	4.1
0.9	0.5	0.0	3.0
0.0	1.2	1.3	0.0
0.6	1.1	1.5	0.5

Chi-square = 16.3 on 9 df.

P > 0.05

Therefore H_o applies

b) CHI-SQUARE ANALYSIS OF HOST VARIABLES (X AXIS) WITH HEMIURID METACERCARIA INFECTION LEVELS (Y AXIS)

i) Metacercariae infection with length

Observed frequencies

20	3	5	13	41
9	17	19	27	72
19	25	25	18	87
11	20	8	2	41
59	65	57	60	241

Expected frequencies

10.0	11.1	9.7	10.2
17.6	19.4	17.0	18.0
21.3	23.5	20.6	21.7
10.0	11.1	9.7	10.7

Contributions to Chi-square

10.0	5.9	2.3	0.8
4.2	0.3	0.2	5.0
0.2	0.1	1.0	0.6
0.1	7.2	0.3	6.6

Chi-sqare = 44.7 on df.

P = 0.000003

Therefore H_1 applies

ii) Metacercariae infection with weight

Observed frequencies

20	1	9	11	41
11	15	18	28	72
15	30	20	22	87
8	21	11	1	41
54	67	58	62	241

Expected frequencies

9.2	11.4	9.9	10.5
16.1	20.0	17.3	18.5
19.5	24.2	20.9	22.4
9.2	11.4	9.9	10.5

Contributions to Chi-square

12.7	9.5	0.1	0.0
1.6	1.3	0.0	4.8
1.0	1.4	0.0	0.0
0.2	8.1	0.1	8.6

Chi-square = 49.57 on 9 df.

P = 0.0000001

Therefore H_1 applies

iii) Metacercariae infection with age

Observed frequencies

21	12	8	41
25	31	16	72
44	33	10	87
31	9	1	41
121	85	35	241

Expected frequencies

20.6	14.5	5.6
36.0	25.4	10.4
43.7	30.7	12.6
20.6	14.5	5.6

Contributions to Chi-square

0.0	0.4	1.0
3.4	1.2	3.0
0.0	0.2	0.5
5.3	2.1	3.8

Chi-square = 20.8 on 6 df.

P = 0.005

Therefore H_1 applies

iv) Hemiurid metacercariae with sex

Observed frequencies

10	17	27
23	42	65
34	42	76
19	16	35
86	117	203

Expected frequencies

11.4	15.6
27.5	37.5
32.2	43.8
14.8	20.2

Contributions to Chi-square

0.2	0.1
0.7	0.5
0.1	0.1
1.2	0.9

Chi-square = 3.823 on 3 df.

$P > 0.05$

Therefore H_o applies

v) Hemiurid metacercariae with gonad weight

Observed frequencies

10	6	4	0	20
11	11	30	5	57
19	14	32	4	69
19	4	8	0	31
59	35	74	9	177

Expected frequencies

6.7	4.0	8.4	1.0
19.0	11.3	23.8	2.9
23.0	13.6	28.9	3.5
10.3	6.1	13.0	1.6

Contributions to Chi-square

1.0	0.2	2.3	1.0
3.4	0.0	1.6	0.4
0.7	0.0	0.3	0.1
7.3	0.7	1.9	1.6

Chi-square = 22.59 on 9 df.

$P < 0.01$

Therefore H_1 applies

vi) Hemiurid metacercariae with Gonad weight as % body weight

Observed frequencies

10	6	2	3	21
10	16	16	16	58
21	8	20	18	67
16	5	7	3	31
57	35	45	40	177

Expected frequencies

6.8	4.2	5.3	4.7
18.7	11.5	14.7	13.1
21.6	13.2	17.0	15.1
10.0	6.1	7.9	7.0

Contributions to Chi-square

1.5	0.8	2.1	0.6
4.0	1.8	0.1	0.6
0.0	2.1	0.5	0.5
3.6	0.2	0.1	2.3

Chi-square = 21.04 on 9 df.

$P < 0.01$

Therefore H_1 applies

vii) Hemiurid metacercariae infection with liver weight

Observed frequencies

13	1	4	6	24
10	10	17	26	63
12	19	22	20	73
8	15	8	5	36
43	45	51	57	196

Expected frequencies

5.3	5.5	6.2	7.0
13.8	14.5	16.4	18.3
16.0	16.8	19.0	21.2
7.9	8.3	9.4	10.4

Contributions to Chi=square

11.1	3.7	0.8	0.1
1.0	1.4	0.0	3.2
1.0	0.3	0.5	0.1
0.0	5.4	0.2	2.8

Chi-square = 31.74 on 9 df.

$P < 0.0005$

Therefore H_1 applies

viii) Hemiurid metacercariae infection with liver as % body weight

Observed frequencies

4	8	5	6	23
15	20	18	12	65
21	23	18	12	74
9	4	10	11	34
49	55	51	41	196

Expected frequencies

5.8	6.5	6.0	4.8
16.3	18.2	16.9	13.6
18.5	20.8	19.3	15.5
8.5	9.5	8.8	7.1

Contributions to Chi-square

0.5	0.4	0.2	0.3
0.1	0.2	0.1	0.2
0.3	0.2	0.1	0.8
0.0	3.2	0.2	2.1

Chi-square = 8.847 on 9 df.

$P > 0.05$

Therefore H_o applies

ix) Hemiurid metacercariae infection with condition factor

Observed frequencies

17	9	7	8	41
8	25	18	21	72
16	29	21	21	87
10	10	11	10	41
51	73	57	60	241

Expected frequencies

8.6	12.4	9.7	10.2
15.2	21.8	17.0	18.0
18.4	26.4	20.6	21.6
8.6	12.4	9.7	10.2

Contributions to Chi-square

8.2	0.9	0.8	0.5
3.4	0.5	0.1	0.5
0.3	0.3	0.0	0.0
0.2	0.5	0.2	0.0

Chi-square = 16.5 on 9 df.

$P > 0.05$

Therefore H_o applies

x) Hemiurid metacercariae infection with total lipid weight of liver

Observed frequencies

2	4	0	0	6
12	13	2	4	31
24	9	5	2	40
2	3	1	1	7
40	29	8	7	84

Expected frequencies

2.9	2.1	0.6	0.5
14.8	10.7	3.0	2.6
19.0	13.8	3.8	3.3
3.3	2.4	8.7	0.6

Contributions to Chi-square

0.3	1.8	0.0	0.5
0.5	0.5	0.3	0.8
1.3	0.3	0.4	0.5
0.5	0.1	0.2	0.3

Chi-square = 10.22 on 9 df.

$P > 0.05$

Therefore H_o applies

xi) Hemiurid metacercariae infection with total lipid weight as % weight of liver

Observed frequencies

2	1	2	1	6
8	7	9	5	29
19	6	9	8	42
0	1	3	3	7
29	15	23	17	84

Expected frequencies

2.1	1.1	1.6	1.2
10.0	5.2	8.0	6.0
14.5	7.5	11.5	8.5
2.4	1.2	2.0	1.4

Contributions to Chi-square

0.0	0.0	0.1	0.0
0.4	0.6	0.1	0.1
1.4	0.3	0.5	0.0
2.4	0.1	0.6	1.8

Chi-square = 8.55 on 9 df.

$P > 0.05$

Therefore H_o applies

xii) Microgemma infection with Hemiurid metacercariae infection

Observed frequencies

11	6	5	2	24
13	18	18	13	62
11	27	37	13	88
6	21	27	13	67
41	72	87	41	241

Expected frequencies

4.1	7.2	8.7	4.1
10.5	18.5	22.4	10.5
15.0	26.3	31.8	15.0
11.4	20.0	24.2	11.4

Contributions to Chi-square

11.7	0.2	1.5	1.1
0.6	0.0	0.9	0.6
1.1	0.0	0.9	0.3
2.6	0.0	0.3	0.2

Chi-square = 21.89 on 9 df.

$P = 0.009$

Therefore H_1 applies

c) CHI-SQUARE ANALYSIS OF HOST VARIABLES (Y AXIS) WITH SEASON (X AXIS)

i) Total Lipid % / season

Observed frequencies

15	0	1	10	26
6	0	8	1	15
7	8	10	1	26
2	5	7	0	14
30	13	26	12	81

Expected frequencies

9.6	4.2	8.3	3.9
5.6	2.4	4.8	2.2
9.6	4.2	8.3	3.8
5.2	2.2	4.5	2.1

Contributions to Chi-square

3.0	4.2	6.4	9.8
0.0	2.4	2.1	1.2
0.7	3.6	0.3	2.1
1.9	3.4	0.9	2.1

Chi-square = 44.199

$P < 0.001$

Therefore H_1 applies

ii) Condition factor / season

Observed frequencies

8	20	5	18	51
8	17	20	30	75
16	13	5	20	54
18	8	21	14	61
50	58	51	82	241

Expected frequencies

10.6	12.3	10.8	17.4
15.6	18.0	15.9	25.5
11.2	13.0	11.43	18.3
12.7	14.7	12.9	20.7

Contributions to Chi-square

0.6	4.9	3.1	0.0
3.7	0.1	0.9	0.8
2.1	0.0	3.6	0.14
2.3	3.0	5.1	2.17

Chi-square = 32.326 df.9

$P < 0.001$

Therefore H_1 applies

iii) Liver weight / season

Observed frequencies

12	10	10	24	66
20	12	12	8	52
20	12	7	11	50
7	16	4	11	38
59	50	33	64	206

Expected frequencies

18.9	16.0	10.6	20.5
14.9	12.6	8.3	16.2
14.3	12.1	8.0	15.3
10.9	9.2	6.1	11.8

Contributions to Chi-square

2.5	2.3	0.0	8.9
1.3	0.0	1.6	4.1
2.3	0.0	0.1	1.32
1.4	5.0	0.7	0.05

Chi-square = 31.614

$P < 0.001$

Therefore H_1 applies

d) CHI-SQUARE ANALYSIS OF SEX (X AXIS) WITH AGE (Y AXIS)

i) Sex with length

Observed frequencies

16	23	39
38	32	64
26	31	57
17	44	61
97	130	227

Expected frequencies

16.7	22.3
27.3	36.7
24.4	32.6
26.0	34.9

Contributions to Chi-square

0.0	0.0
3.0	0.7
0.1	0.1
4.8	1.9

Chi-square = 10.553 on 3 df.

$P < 0.025$

Therefore H_1 applies

ii) Sex with age

Observed frequencies

43	35	8	86
47	44	27	118
90	79	35	204

Expected frequencies

38	33.5	14.4
52.1	45.7	20.24

Contributions to Chi-square

.65	0.6	2.84
.48	0.6	2.25

Chi-square = 6.87 on 3 df.

$P < 0.05$

Therefore H_1 applies

e) CHI-SQUARE ANALYSIS OF SEX/AGE COMBINATIONS (X AXIS) WITH INFECTION (Y AXIS)

i) With *Microgemma* infection

Observed frequencies

10	22	19	22	4	19	96
35	29	14	25	4	18	125
45	51	33	47	8	27	211

Expected frequencies

20.5	23.2	15.0	21.4	3.6	12.3
24.5	27.8	18.0	25.6	4.4	14.7

Contributions to Chi-square

5.4	0.1	1.1	0.0	0.0	3.7
4.5	0.1	0.9	0.0	0.0	3.1

Chi-square = 18.72 on 5 df.

P = 0.002

Therefore H_1 applies

ii) With Hemiurid metacercariae infection

Observed frequencies

8	19	13	19	4	14	77
37	32	20	28	4	13	134
45	51	33	47	8	27	211

Expected frequencies

16.4	18.6	12.0	17.2	2.9	9.9
28.6	32.4	21.0	29.8	5.1	17.1

Contributions to Chi-square

4.3	0.0	0.1	0.2	0.4	1.7
2.5	0.0	0.0	0.1	0.2	1.0

Chi-square = 10.63 on 5 df.

P 0.05

Therefore H_o applies